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EFFECTS OF SOME PROCESSING TREATMENTS ON CHEMICAL, NUTRITIONAL AND FUNCTIONAL PROPERTIES OF RHUBARB STALK

by

SIRIYUPA NETRAMAI

A thesis submitted to the Faculty of Graduate Studies and Research

in partial fulfillment of requirements for the degree of

MASTER OF SCIENCE in Food Science and Technology

Department of Agricultural, Food and Nutritional Sciences

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FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled EFFECTS OF SOME PROCESSING TREATMENTS ON CHEMICAL, NUTRITIONAL AND FUNCTIONAL PROPERTIES OF RHUBARB STALK submitted by SIRIYUPA NETRAMAI in partial fulfillment of the requirements for the degree of MASTER OF SCIENCE in Food Science and Technology.

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Date: August 8, 2003

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ABSTRACT

Effects of some processing treatments on important properties of rhubarb pulp and juice were studied. Freezing rhubarb stalks before juice separation decreased soluble dietary fiber (SDF), total anthocyanins (TACN), protein and fat contents as well as WHC and BSBC of rhubarb pulp. Blanching rhubarb pulp decreased its IDF, WHC and BSBC, but increased its SDF, anthocyanin content and antioxidant capacity. The increased antioxidant capacity was attributed partly to Maillard's reaction products (MRPs). Air- and drum-drying caused the decrease in fiber and TACN contents, and WHC and BSBC, as compared to freeze-drying. However, the two pulps had higher antioxidant capacity, most likely due to the formation of MRPs.

Vacuum evaporation reduced SDF and TACN in rhubarb juice, but anthocyanin stability during storage was better in concentrated juice than raw juice. Rhubarb pulp and raw juice showed no antimicrobial activity against selected pathogens. Concentrated juice showed antimicrobial property, probably due mainly to its acidity.

To Dad, Mom and Pa

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List of Abbreviations

AC	Antioxidant capacity
ADB	Air-dried blanched
ADUB	Air-dried un-blanched
AMC	Antimicrobial capacity
B	Blanched
BSBC	Bile-salt binding capacity
DDB	Drum-dried blanched
DDUB	Drum-dried un-blanched
DF	Dietary fiber
FDB	Freeze-dried blanched
FDRP	Freeze-dried rhubarb pulp
FDUB	Freeze-dried un-blanched
FRAP	Ferric reducing antioxidant power
FsRP	Fresh rhubarb pulps
FzRP	Frozen rhubarb pulps
IDF	Insoluble dietary fiber
LDL	Low-density lipoprotein
MRPs	Maillard's reaction products
ORAC	Oxygen radical absorption capacity
PA	Protective activity
PME	Pectin methyl esterase

SDF	Soluble dietary fiber
SE	Squalene epoxidase
TACN	Total anthocyanins
TDF	Total dietary fiber
TE	Trolox equivalent
TEAC	Trolox equivalent antioxidant capacity
TSS	Total soluble solid
UB	Un-blanched
WHC	Water holding capacity

CHAPTER 1

General Introduction and Research Objectives

Rhubarb stalk consists of two major components, i.e. pulp and juice. Dry rhubarb pulp, which consists principally of insoluble and soluble dietary fiber, has high water holding capacity (WHC) and the ability to bind bile salt (Goel *et al.*, 1997 and Ooraikul *et al.*, 1993). Its ability to lower an average of 8% of serum cholesterol concentration, especially low-density lipoprotein (LDL), in hypercholesterolemic men fed with 27 g of ground rhubarb stalk fiber per day for 4 weeks has been reported by Goel *et al.* (1997). The cholesterol-lowering effect of rhubarb could be attributed to its ability to inhibit squalene epoxidase (SE) enzyme, since regulation of the level of SE enzyme has a clinical importance in the modulation of cholesterol biosynthesis (Abe *et al.*, 1995 and Abe *et al.*, 1998). Rhubarb has also been shown to modulate sugar absorption in rats with diabetic nephropathy (Yokozawa *et al.*, 1997).

Rhubarb fiber produced with a fiber separation method developed by Ooraikul *et al.* (1993) has been used as an ingredient in the development of several products such as meat analogs and vegetarian jerky to improve their firmness and chewability (Atapattu, 1993; Ooraikul *et al.*, 1993; and Shum, 1998).

Rhubarb juice has been demonstrated to have anti-browning and antioxidant activities. Oszmianski *et al.* (1994) reported that addition of 2 - 3% of rhubarb juice to apple pulp showed the same effects as the application of 0.5% ascorbic acid. It effectively inhibited enzymatic oxidation, and protected phenolics and colour from degradation and browning without adversely affecting the flavor of apple pulp. Interestingly, the product so treated showed better sensory characteristics, and its color was less sensitive to storage

conditions compared to the samples treated with ascorbic acid. The use of rhubarb juice as antibrowning agents in fresh-cut apple slices showed the same trend as that in unclarified apple juice (Son *et al.*, 2000).

However, the high oxalic acid content in rhubarb may be a limiting factor in its use, since oxalic acid is the most effective inhibitor of calcium absorption. Over-consumption of oxalate may lead to kidney stone formation (Hesse *et al.*, 1993 and McCombs and Gershoff, 1972).

A number of developments have been made to produce a variety of food products from rhubarb stalks, e.g. rhubarb ice cream (Harra, 1990), rhubarb candies, rhubarb catsup (Ooraikul *et al.*, 1993) and rhubarb jam (Yoshida *et al.*, 1996). Concentrated rhubarb juice has been used to produce jelly, topping and beverages (Treptow, 1985 and Ooraikul *et al.*, 1993).

Rhubarb is grown widely in North America, Northern Europe and Australia. They are normally harvested twice a year, in mid-summer and fall. In North America, rhubarb stalk is used commercially as pie filling or for juice production. To extend the processing season of raw rhubarb year-round, rhubarb stalks are normally cleaned, trimmed, cut and frozen. Rhubarb juice may be separated from the pulp by pressing fresh or frozen and thawed stalks, with or without blanching. If rhubarb pulp and juice are to be used as food ingredients, for functional or nutritional purposes, the pulp may be dried and ground and the juice may be thermally concentrated so that they can be stored over a long period.

However, processing treatments involving heat or cold may impart certain changes to the chemical, functional and nutritional characteristics of rhubarb stalk and its

products. For example, Collins and Marangoni (1998) have reported a decrease in vitamin C and Van Buren (1979) has reported a change in dietary fiber (DF) fractions during storage at low temperature.

Thermal treatments can induce a variety of changes to plant materials. Ishi *et al.* (1990) reported that anthocyanin pigments were adversely affected by heat, while Kok-Sian and Ishak (1991) showed that blanching might enhance the stability of the same pigments. Thermal degradation of antioxidants or prooxidants such as anthocyanins may lead to the decrease of antioxidant activity. However, the same thermal processes may cause a formation of new antioxidant compounds leading to an overall increase in antioxidant capacity of the product (Eriksson and Na, 1995; Nicoli *et al.*, 1995 and 1997). Therefore, the effects of various processing treatments on plant materials such as rhubarb stalks may be positive, negative or neutral, depending on factors such as composition of the material, types and conditions of processing used, and post-processing treatments such as packaging and storage.

Much is known about the functional properties and nutritional characteristics of rhubarb stalks. However, the effects of processing on these attributes have not been adequately investigated or documented. Therefore, the general objective of this study was to investigate the effects of some processing treatments on important attributes of rhubarb stalks. Specifically, the objectives were:

1. To study the effect of freezing, blanching, and different types of drying, on chemical, nutritional and functional properties of rhubarb pulp. The properties studied included insoluble dietary fiber (IDF), soluble dietary fiber (SDF), total dietary fiber (TDF), total anthocyanins (TACN), protein,

fat and ash contents, water holding capacity (WHC), bile salt binding capacity (BSBC) and antioxidant capacity (AC).

2. To study the effects of vacuum evaporation on IDF, SDF, TDF, TACN, protein, fat and ash contents and AC of rhubarb juice.
3. To observe the relationship between the chemical changes and nutritional and functional properties, especially water holding, bile salt binding and antioxidant capacities, of rhubarb pulp and juice.
4. To determine the antimicrobial capacity (AMC) of rhubarb.

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CHAPTER 2

Literature Review

2.1 Rhubarb

Rhubarb (*Rheum* sp.), a perennial herb, is a member of the buckwheat family, Polygonaceae. Rhubarb was not accepted as a food in the United States until late 1700s, and only after creative experimentations with its culinary attributes. In 1947, rhubarb was legally considered as a fruit, even though botanically, it is a vegetable.

There are many species of plant which are called rhubarb. Rhubarb varieties are classified as red or green (Figures 2.1 and 2.2). Green types are further differentiated as green and speckled (pink). The different names may be assigned to the same variety in different regions or countries as people move planting material from one location to another (Thuesen, 1982; and www1,2).

Figure 2.1: Red rhubarb stalks (www3)

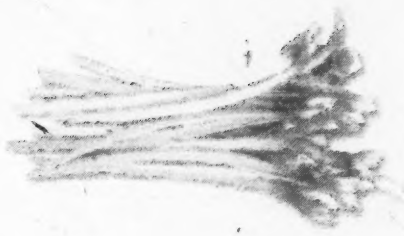

Figure 2.2: Green rhubarb stalks (www3)

Although there are several different varieties of rhubarb grown all over the world and used in a variety of cooking preparations, one characteristic consistent with all rhubarb is the toxicity of the leaves and roots. The rhubarb leaves contain high amounts of oxalic acid, a toxic and potentially deadly poison. Only the stems (stalks) are edible (www4).

2.2 Important chemical, nutritional and functional attributes of rhubarb stalk

2.2.1 Chemical composition

Typical composition of rhubarb stalk is given in Table 2.1.

Nutritionally, rhubarb is low in calories and very acidic (pH 3.1). The acid may be offset by the addition of sugar in food preparation, which also increases the calorie count. Although, the calcium contained in rhubarb is high, its supply is bound by oxalic acid and so it is not easily absorbed by the body. Rhubarb cannot be counted upon as a source of dietary calcium (www4).

Table 2.1: Nutritional content of raw rhubarb (Scientific Name: *Rheum rhabarbarum*) (www5)

Nutrient	Value per 100 g of edible portion (g)
Water	93.61
Protein	0.90
Total lipid (fat)	0.20
Carbohydrate, by difference	4.54
Fiber, total dietary	1.8
Ash	0.76
Vitamin C	0.008

Rhubarb stalk contains 93 – 95% (w/w) water. Other major components are dietary fiber, minerals and organic acids. Rhubarb is considered a good source of dietary fiber. On dry basis, the stalk has 74% dietary fiber (Ooralkul *et al.*, 1993). The fiber in the stalk consists of long strands of cellulosic material coated with pectic substances and is relatively easy to separate into individual strands.

2.2.2 Dietary fiber

For analytical purposes, the term dietary fiber (DF) refers mainly to non-starch polysaccharides and lignin in the diet (Southgate, 1976). The composition of plant cell walls, the main source of DF, depends on the stage of maturity at which the plant organ is harvested.

The main components of DF polymers in fruits and vegetables are pectic substances, cellulose, hemicellulosic polymers, lignin, some proteins and phenolics associated with complex polysaccharides.

Soluble fiber has effects on lipid and glucose metabolism, whereas insoluble fiber has been shown to increase faecal bulk due to its physiological properties (Svanberg *et al.*, 1995).

2.2.2.1 Cell wall

Cell wall, a major source of DF, has a highly ordered structure with alternating layers of cellulose microfibrils embedded in a matrix of hemicelluloses and pectic substances. The central region of the wall, the middle lamella, which acts as cementing layer, is rich in pectins. The whole wall may be further strengthened by layers of secondary thickening which may or may not be lignified.

In the living plant there is an osmotic pressure generated within the cell sap which gives rise to the so-called turgor pressure which maintains the protoplast firmly pressed against the cell wall and hence keeps the tissue firm or turgid. In living plants, which are subjected to stress from water loss or deficiency, the tissues frequently become flaccid and this is related to the loss of turgor pressure within the cell. This process is known as plasmolysis which often observed during the processing of fruits and vegetables.

Prior to harvesting, the hemicellulose level increases and the pectin content decreases. During storage, however, hemicellulose decreases and pectin increases. These are due to the changes within protoplast and cell wall (Jewell, 1979).

2.2.3 Anthocyanins

Rhubarb exhibits a wide range of colors from light green to red, with the development of the red pigment being most noticeable in the stalks towards maturity (Wrolstad and Heatherbell, 1968). Special interest is usually paid to the anthocyanin

content since petiole color influences consumer preferences and determines the use in processing (Rumpunen and Henriksen, 1999).

Anthocyanin, the natural colorant, is a glycoside form of anthocyanidins, the benzopyrylium and flavylium salts (Belitz and Grosch, 1987). With few exceptions the known natural anthocyanins are 3- or 5-monoglycosides or 3,5-diglycosides of the anthocyanidins (Jurd, 1969). The color of anthocyanins is dependent on pH due to the existence of four different structures in aqueous solutions (Brouillard and Delaporte, 1977). The four structures have different colors and individually vary in concentration throughout the pH scale with the red flavylium cation as the dominant structure in acidic environment. The exact color of the solution depends on the pH value (Mazza and Brouillard, 1987)

Chichester (1972) discovered that at pH 0.71, anthocyanin is intense red and the pigment is primarily in flavylium cation form. As the pH value rises, the pigment changes to the anhydro-base and then to the carbinol which is a colorless compound.

Rhubarb contains anthocyanins, water-soluble pigments, in the epidermal layer of the stalk, particularly at the base, and in the roots. The anthocyanins of rhubarb stalks were first identified by Gallop (1965) as cyanidine-3-glucoside and cyanidine-3-rutinoside. The same two pigments were confirmed by Blundstone and Crean (1966); and Hetmanski and Nybom (1968). Wrolstad and Heatherbell (1968) also reported that the major anthocyanin found in rhubarb (*Rheum rhaponticum,* Canada Red) was cyanidin-3-glucoside (87%), one of the most unstable anthocyanins (Rommel, and Wrolstad, 1993), and cyanidin-3-rutinoside (13%) was also found in the sample. The third anthocyanin (present in trace amounts) was also reported and later identified as a bioside of cyanidin.

The investigation of some rhubarb cultivars, such as 'Sunrise', 'Valentine', and 'German Wine' rhubarb by Fuleki (1969) found the levels of cyanidin-3-glucoside and cyanidin-3-rutinoside between 33-58% and 41-66%, respectively.

The range of anthocyanins in the petiole juice of rhubarb was found to be 0-154 mg/kg of fresh weight. Negative correlations were found between the anthocyanin content and some growth characteristics, e.g. leaf size and petiole width (Rumpunen and Henriksen, 1999; and Thuesen, 1982).

Anthocyanins were reported to exhibit antioxidant activity and inhibit low-density lipoprotein (LDL) oxidation as well as show anti-inflammatory activity (Kalt *et al.*, 1999; Prior *et al.*, 1998; and Wada and Ou, 2002). The linear relationship (r^2) between total antioxidant activity (using ORAC assay) and anthocyanins was found to be 0.932. This result was in agreement with the investigation by Prior *et al.* (1998). The antioxidant capacity obtained from FRAP assay showed a linear relationship with anthocyanin in Roselle extract.

Factors affecting the color and stability of anthocyanins include structure, concentration, pH, temperature, light, co-pigments, self-association, metallic ions, enzymes, oxygen, ascorbic acid, sugars and their degradation products and proteins (Francis, 1989; Henry, 1996; and Mazza and Miniati, 1993) and the pigment degradation was reported to be a first-order reaction (Sapers *et al.*, 1981).

Wrolstad *et al.* (1970) suggested that the anthocyanin degradation reactions could be enzymic and/or chemical. There are several enzyme systems researchers claimed to accelerate the destruction of anthocyanin pigments, such as polyphenoloxidase,

glycosidase and peroxidase (Cash *et al.*, 1976; Grommeck and Markakis 1964; Huang, 1955; and Peng and Markakis, 1963).

The activity of enzyme β-glucosidase in monomeric anthocyanin, such as cyanidin-3-glucoside and cyanidin-3-rutinoside degradation was discovered by Wightmand and Wrolstad (1996). Anthocyanins with β-glucosidic linkages are the most prevalent pigments in anthocyanin-colored fruit. The β-glucosidase would cleave off the glucose unit producing the unstable aglycons, and α-rhamnosidase activity could cleave off the rhamnose unit in cyaniding-3-rutinoside.

Markakis (1974) showed that the rate of ascorbic acid oxidation also influenced the anthocyanin loss in strawberry products.

2.2.4 Water holding capacity

The water holding capacity (WHC) of a fiber preparation is a measure of its ability to immobilize water within its matrix (Selvendran *et al.*, 1987).

When DF is placed in water, the exposed regions of fiber polymers absorb water molecules. Some water is held within the hydrated wall matrix and some fills the dehydrated as well as partially broken cells. Water is also held in the interstitial spaces as trapped water. On finely comminuted fiber preparations, more DF polymers would be exposed to absorb water, but the number of dehydrated cells which can imbibe water would decrease as would the interstitial spaces available for the retention of water. Water outside the matrix, which can be removed by filtration or by centrifugation, is called free water.

When a partially soluble polysaccharide, e.g., pectin, is placed in water, the water penetrates amorphous regions and binds to available sites. This leads to the reduction in interpolysaccharide associations. Parts of the polysaccharide become solvated, and other regions associate with each other through formation of Ca^{2+} bridges and hydrogen bonding, forming a loose gel network.

Linear polysaccharides, e.g., cellulose microfibrillar structure, do not dissolve in water because the polysaccharide chains associate with one another by intermolecular hydrogen bonds. However, the glucan chains at the surface of the cellulose microfibrils hydrogen bond with water and can retain some water.

The methods to determine WHC usually involve saturating the fiber with excess water and removing the free water by centrifugation or filtration.

Several factors that influence WHC of DF are particle size of the DF sample, the chemical composition of the fiber and the nature of the associated compounds, and possibly the pH (Robertson and Eastwood, 1981a,b; Eastwood *et al.*, 1983; and Eastwood and Passmore, 1983).

Mongeau and Bassard, (1982) suggested that the difference in WHC of breakfast cereals and brans did not seem to be due to compositional change of their DF, but to structural changes of the fiber matrix during grinding. This is in agreement with the results of Soest (1981), that grinding of wheat bran decreases WHC by changing the physiological characteristics of the fiber. Since aleurone cells in bran are like holes in a sponge, they absorb water. When particle size is reduced, fewer pores and voids remain to hold water, thus the WHC decreases (Schaller, 1978).

2.2.5 Bile salt binding capacity

Fiber preparations have been shown their capability of binding bile salt (Eastwood and Hamilton, 1968; and Eastwood *et al.*, 1976). It has been suggested that DF binds bile salts and other steroids in the intestine and therefore inhibits cholesterol absorption (Balmer and Zilversmit, 1974; and Eastwood and Passmore, 1983). The binding was shown to be dependent on time of exposure and pH of the medium. Maximum binding occurred at acidic pH, and the binding was considerably reduced at alkaline pH.

The hypolipidemic effect of rhubarb fiber (*Rheum rhaponticum*) was reported by Goel *et al.* (1999). The rhubarb stalk is effective in lowering serum cholesterol concentrations, especially low-density lipoprotein (LDL) cholesterol, in hypercholesterolemic men (Goel *et al.*, 1997).

Jonsson (1969) reported about the bile salt binding determination using the *in vitro* method introduced by SIK (Swedish Institute for Food Research) that the soluble fibers bound considerable amounts of bile salts. He listed bile salt binding capacity of soluble fibers from various sources in the following descending order: pectin (degree of esterification = 73), beta-glucan (from oat bran), guar gum and pectin (degree of esterification = 64). He also reported that insoluble fiber and cellulose bound only negligible amounts of bile salts.

Mongeau and Brassard (1982) proved that the physical change, i.e., smaller particle size caused the decrease in wheat bran's bile salt binding capacity *in vitro*. This probably implies that the physical state of the fiber is related to its physiological role such

as WHC and binding of organic compounds, which are associated with the nutritional characteristics (Schneeman, 1986).

2.2.6 Antioxidant activity

Generally, antioxidants can be classified into two mechanistic categories: preventive antioxidants which inhibit the formation of reactive oxygen species, such as enzyme systems like catalase and peroxidase, and chain-breaking antioxidants, such as vitamins C and E, polyphenols, etc. For the latter type, there are two possible pathways. The first pathway involves a hydrogen atom transfer (HAT) reaction, where the oxygen radical abstracts hydrogen from the antioxidant yielding a stable antioxidant radical. The second pathway in which antioxidant deactivates free radicals is called single electron transfer (SET) reaction (Ou *et al.*, 2002). According to the results provided by Wright *et al.* (2001), in most cases HAT will be dominant.

The antioxidant capacity in plant can be ascribed to the presence of compounds occurring naturally in plants, such as phenols, ascorbic acid, carotenoids, etc. (Larson, 1988). Recent evidence indicated that antioxidant capacity in plants is probably attributable principally to their flavonoid content (Kalt *et al.*, 1999; Chu *et al.*, 2000; and Vinson *et al.*, 1998).

Rhubarb juice was reported by Thakur *et al.* (1989) as a fairly good source of vitamin C. Munzuroglu *et al.* (2000) discovered that the amount of antioxidant vitamins, i.e. vitamins A, E and C, in rhubarb (*Rheum ribes L.*) grown at high attitudes was significantly higher than that in the plants grown at low altitudes.

Phenolic compounds and flavonoids may provide long-term protection against a number of chronic diseases (Hertog, 1994). The important flavonoids are anthocyanins, flavan-3-ols, etc. (Haslam, 1998). In varying degrees, these compounds show antioxidant capacities *in vitro*, scavenging $O_2^{\cdot-}$, $OH^{\cdot}$, and $ROO^{\cdot}$ (Van Acker *et al.*, 1996), inhibiting lipid peroxidation (Vinson and Dabbagh, 1998), and protecting low-density lipoproteins against oxidation (Aviram *et al.*, 1997). They can also inhibit platelet aggregation (Renaud and De Lorgeril, 1992) and enhance vasodilation (Fitzpatrick *et al.*, 1993).

Rhubarb juice has been reported to have a superior antioxidant capacity over ascorbic and citric acids when used as an antioxidant in carrot juice (Oszmianski and Gorska, 2002). It has also been used as an antibrowning agent on fresh-cut apple slices (Son *et al.*, 2000). Tong *et al.* (1995) suggesting that the antibrowning effect of rhubarb juice may be largely due to the presence of oxalic acid in the juice, since the antibrowning activity was markedly decreased when oxalic acid was removed from the sample.

2.2.7 Antimicrobial capacity

The antimicrobial ability of rhein, one of the common anthraquinones in most of the rhubarb species, was observed against some microorganisms such as *Escherichia coli*, *Bacillus subtilis* and *Clostridium perfringens* (Ahmad *et al.*, 1998). Chopra and Nayyar (1978) also reported rhubarb's antibacterial activity against *Staphylococcus aureus*, *Brucella abortus* and *E. coli*.

2.3 Vegetable preservation

Most plants have limited harvest periods and fresh shelf life. To extend their availability, some form of storage and preservation is needed. A variety of preservation methods exist, each of which results in an extended shelf life of fresh or processed plant materials.

2.3.1 Freezing storage

Freezing has been used for long term preservation of many plants (Reid, 1983). Freezing involves the use of low temperature under which many chemical reactions and microbial activities slow down. There possibly are some changes in membrane and organelle properties that produce altered metabolic pathways. Chilling can cause quality loss in food products, called chilling damage or chilling injury (Lyons, 1973; and Wilson, 1987). If chilling damage is not present, refrigeration can lead to a significant extension of product shelf life.

Freezing implies phase change. The aqueous component of the tissue is present in at least two phases, i.e. ice and the increasingly concentrated solution. Since some water has separated out as ice, the remaining liquid will increase in concentration. The process, which attempts to equalize the concentration of the solutions on either side of the barrier, is known as osmosis. The presence of ice and the increase in the concentration of solutes can have significant effects on some characteristics of frozen products (Reid, 1983; and Brown, 1979).

There are two basic types of freezing process, i.e. fast freezing and slow freezing.

In fast freezing, once the process is applied, ice begins to form in the space between cells. There is insufficient time to remove water from cell through osmosis. The cell contents undercool and seed, so ice is also formed within the cell in fast freezing.

For slow freezing, there is enough time to remove a large amount of water from cells. The concentration of the cell content increases rapidly, thus prevents the cell content from being significantly undercooled. Ice does not form within the cell.

The transfer of large amount of water from cell to the external space in slow freezing can result in cell shrinkage, which can damage the membranes (Meryman, 1971 and Steponkus, 1984). A considerable amount of water translocates. Due to cell wall damage during the freezing process, the water does not return to the cells during thawing, but rather becomes drip loss.

Freezing materials in large cold room can be ineffective. The heat transfer mechanism in cold air is through convection. Packing the products in large containers or freezing the products in a high-capacity cooling unit may lead to slow freezing, due to heat transfer limitations (Persson and Londahl, 1993).

Reid (1983) suggested that freezing rhubarb stalk requires no special treatment; however, a short blanching can probably extend its storage life. The storage life of frozen rhubarb stalks at -18°C was reported to be at least 6 months.

2.3.2 Drying

A number of processing methods give vegetable products higher storage stability and readily transformed them into a consumable form. The preservation of foods by

drying is one of the oldest methods used by man. Dehydrated vegetables are light, air and moisture sensitive, so they may require a suitable packaging.

Prescott and Sweet (1919) defined dehydration as "the process of removal of surplus water without destruction of cellular tissues, or impairment of the energy values."

There are four types of dryers used to dehydrate rhubarb pulps in this experiment, i.e. freeze dyers, drum dryer, bin dryer and fluidized bed dryer.

2.3.2.1 Freeze dryer

This process is usually used to preserve the relatively labile substances. The material to be dried is first frozen, and then bled of its water content under a high vacuum. Ice from the frozen product is sublimed by the application of heat without melting the product.

Dehydration by freeze drying gives the highest quality products with a spongy and porous structure which is readily rehydratable (von Loesecke, 1955). The low processing temperatures and the rapid transition of the product to dry form minimizes the extent of various degradative reactions associated with other drying methods. Hence, freeze-dried samples were used as a control in every experiment of this project.

2.3.2.2 Drum dryer

Drum drying can be either atmospheric or vacuum, consisting of steam-heated drums to which the material to be dried is applied by different feeding devices depending on the type of material being dried. The drums can be single or double. When operated, the drums revolve, and before they have made one complete revolution, the material is removed by a 'doctor blade', or scraper.

2.3.2.3 Bin dryer

This drying method is widely used as the finishing step in the dehydration of piece-form vegetables. Bin dryers are used to complete the drying operation after most of the moisture has been removed by other techniques. Typically, they reduce the moisture content of a partially dried, 10 to 15% moisture cut vegetable such as onion and cabbage shreds to 3-6% or even lower.

Bin dryer units are usually built with metal or wooden boxes with an air inlet at the bottom.

2.3.2..4 Fluidized bed dryer

In this type of drying, heated air is blown up through the food particles with just enough force to suspend the particles in a gentle motion. Heated air is introduced through a porous plate that supports the bed of particles. The moist air is exhausted at the top.

2.3.3 Blanching

Blanching was introduced as a preparation step for vegetables for drying after World War I (von Loesecke, 1955). Blanching is desirable because it aids in preserving vitamins during drying, improves the color of pigmented vegetables, increases the drying rate and decreases the bacterial population. Since enzymes can be inactivated by heat, the blanched dehydrated vegetables tend to have a longer storage life than the un-blanched products.

There are several blanching methods available, e.g. steam, hot water, and series blanching. The steam blanching was used to blanch rhubarb pulps in this experiment.

Ooraikul *et al.* (1993) experimented on separating long strand rhubarb fiber from rhubarb stalks to be used as a texturizing ingredient in some food products. They blanched rhubarb stalks prior to fiber separation. They found that apart from helping inactivate enzymes, blanching softened the tissue sufficiently, but not excessively to weaken the fibers, so the fiber strands may be separated cleanly from one another while retaining appropriate amount of pectic 'coating' for desirable chewing characteristics. Though fiber separation without blanching is possible, it is found that blanching is necessary to soften the cellulosic fiber and swell the pectic substances for proper coating and chewing quality. Without blanching the fiber is raw and stringy on chewing, and subsequent cooking does not remove these undesirable characteristics.

Blanching also helps remove undesirable flavoring compounds, such as those in cabbages, and the air present in plant tissue, as well as induce shrinkage or softening of the products.

2.3.4 Evaporation

Evaporation is understood as a physical process of concentrating solutions by partial removal of water from them by vaporization. Generally, in the food industry, evaporation is used to pre-concentrate the solution prior to further processing, to reduce the liquid volume for less storage space or to increase the concentration of soluble solids in food materials as an aid to preservation.

The evaporator system normally consists of a heat exchanger to supply heat to the feed, a separator for the vapor to separate from the concentrated liquid phase and a

condenser to effect the condensation of the vapor and its removal from the system (Brennan *et al.*, 1969).

To reduce the heat damage, lowering the pressure at which evaporation takes place is required. This leads to the lowering of the boiling point, especially in cases where heat sensitivity is a factor.

Evaporation darkens the color of food, partly because of the increase in the concentration of solids, but also because the reduction in water activity promotes chemical changes, for example, Maillard browning. As these changes are time and temperature dependent, short operating time and low boiling temperatures produce concentrates with a good sensory and nutritional qualities (Fellows, 1988).

2.3.5 Heat transfer

There are three ways to transmit heat from a hot object to a cold object. Most food engineering applications are combinations of two or three of these mechanisms. These are:

2.3.5.1 Conduction

The internal kinetic energy of a substance is largely due to molecular vibrations when the molecules of the substance, which could be liquid, solid or vapor, are restrained so that no appreciable relative translatory motion occurs among them. If there is temperature difference in the substance, some adjacent molecules have different rates of vibration. The molecule with the higher rate of vibration transfers some of its motion to adjacent molecules with slower rates of vibration. The thermal energy is transferred by the mechanism of conduction from a region of higher temperature to a region of lower

temperature. The process of heat conduction continues until temperature distribution in the substance reaches equilibrium.

2.3.5.2 Convection

The densities of all materials change with changes of temperature. In fluid, i.e., liquids, vapors, and gases, any differences in densities which are caused by temperature differentials lead to mass movement to equalize the density of fluid. Heat transmission through mass flow of fluid is called heat convection.

Convection is essentially a mixing process and any method of increasing the rate of mixing correspondingly increases the rate of convective heat transfer.

2.3.5.3 Thermal radiation

When heat is transmitted between two objects at different temperatures by means of electromagnetic waves the transmission is said to be by thermal radiation. The radiation can occur without the presence of any intermediate substance between the two objects. However radiative heat energy does not usually permeate through liquid or solid due to the energy reflection and absorption by those substances. Most gases, except water vapor and carbon dioxide, do not absorb radiative energy significantly (Fellows, 1988).

2.4 Effects of some processing operations on plant materials

2.4.1 Freezing

The experiment performed by Ancos *et al.* (2000) showed that freezing process did not affect the level of vitamin C and antioxidant capacity of spinach raspberries. These results are in agreement with those of Hong and Ueda (1993) who found that the vitamin C content in strawberries did not markedly change during storage at -20°C for 6

months, but the anthocyanin level was reduced to less than half after the storage. Unlike red radish and red-fleshed potato juices (Rodriguez-Saona *et al.*, 1999), refrigeration (2°C) helps increase their anthocyanin half-life to over a year. Hence, the pigment degradation depends on storage temperature and anthocyanin structure. Collins and Marangoni (1998) found that a 10°C rise in temperature above -18°C causes the degradation factor for vitamin C to increase 6-20 fold.

The storage of onions mimicking home-style storage for 6 weeks resulted in a parallel decrease of total anthocyanins and total antioxidant capacity (Gennaro *et al.*, 2002).

Changes in dietary fiber fractions were also observed. Svanberg *et al.* (1995) discovered the decrease of soluble fiber content and increase of insoluble fiber in various cultivars of carrots. A decrease in solubility of fiber during storage might be due to enzyme pectin methyl esterase (PME) (Van Buren, 1979). In long-term storage, there is a possibility for PME to demethoxylate pectic substances leading to a higher proportion of free carboxyl groups which are able to interact with Ca^{+} ions. Consequently, the solubility of fiber decreases.

2.4.2 Thermal processing

The objectives of vegetable dehydration is to reduce the water content of the plant below the level critical for the growth of microorganisms (12-15%) without being detrimental to important nutrients, and to attempt to preserve flavor, aroma and appearance as well as the ability to regain the original shape (Karel, 1975). However, notable changes are caused by dehydration process. First, there is a concentration of major ingredients, such as proteins, carbohydrates and minerals along with some

chemical changes. Fat is degraded via oxidation, which could lead to odor and flavor losses. Amino compounds and carbohydrates involved in a Maillard reaction yield darker color and new aromas in the products. Vitamin levels may also drop markedly. Losses of original volatile aroma and flavor compounds are expected.

Heat treatment such as blanching can cause damage to cell barrier. This damage renders it unable to support the osmotic process, making molecular translocation of solutes across the barrier much easier.

Mohr (1971) noticed changes associated with freeze-thaw and blanching in rhubarb. He detected a gradual breakdown in the organization of the protoplasmic structure and, in most cases, a rupture of the plasmalemma with subsequent loss of turgor pressure. Some degradation and separation of cell walls was also observed in a number of experiments (Monzini, 1974). A similar response was also observed in the blanching of carrots (Jewell, 1979). Steam blanching produced protoplasmic degradation and the formation of numerous vesicles, but cell wall degradation was minimized compared to water-blanched samples.

In thermal and chemical processing, changes in the cell wall structure, such as splitting along the middle lamella due to modifications of pectins and hemicellulose, are normally observed.

In thermal processes, e.g. cooking, glycosidic linkages in the dietary fiber polysaccharides could be broken (Nyman *et al.*, 1987a,b; and Svanberg *et al.*, 1995). At a moderate degradation the amount of soluble fiber will increase, and there will be an apparent decrease in the total DF content if the decomposition is extensive enough to cause a formation alcohol-soluble fragment.

The depolymerization of fiber polysaccharides is quite important from the nutritional standpoint. A breakage of glycosidic linkages may result in an increase in the fermentability of the fiber in the large intestine (Nyman, 1987; Svanberg *et al.*, 1995; and Bjorck, 1984). As the viscosity is reduced with the cleavage of a few glycosidic linkages (Albersheim *et al.*, 1960), heat treatments may also reduce the fiber's effects on carbohydrate and lipid metabolism.

However, there may also be changes in the fiber, which cannot be determined with recent fiber analysis, e.g., a moderate depolymerization (Plat *et al.*, 1988 and Missiot *et al.*, 1992). This change can lead to nutritional changes, due to the relationship between physical properties, such as viscosity and degree of polymerization.

Effects of heat on DF fractions depend on fiber type and processing methods. For example, fiber analyses in apple, corn, oat bran and soy affected by autoclaving and microwave heating performed by Chang and Morris (1990) showed that autoclaving reduced IDF in apple and TDF in apple and oat bran. Microwave heating lowered TDF in apple and oat bran and IDF in oat bran, but increased SDF in apple, while both treatments decreased SDF in corn.

The cellulose produced by *Acetobacter xylinum* showed reduced WHC in the dried forms. Jimenez *et al.* (2000) found that the WHC of table olive fibers were related to moisture content with the lowest value in dried sample.

Nutrient stability during processing is affected by a number of conditions, including pH, presence of oxygen, light and heat or combinations of these factors (Karel, 1985).

Carvajal *et al.* (1998) reported that around 75% of ascorbic acid is lost during drying and grinding. The loss of ascorbic acid during grinding and drying could be caused either by the sensitivity of vitamin C toward heating (55°C for 48 h and the heat from grinding), or by the capability of vitamin C to act as an antioxidant. The degradation of ascorbic acid in air-dried tomatoes showed the same trend (Lavelli *et al.*, 1999). The oxidation of ascorbic acid can lead to an initially accelerated browning rate, which affects the overall browning occurring during storage in kiwifruit juice concentrate (Wong and Stanton, 1993).

Processing of plants can have a great effect on their antioxidant activity (Gazzani *et al.*, 1998). However, the tendency of its impact on the total antioxidant capacity cannot be predicted due to the fact that during processing, the antioxidant activity can decrease from the loss of antioxidant or the prooxidant formation. On the other hand, the processing can cause structural alterations in the existing antioxidants or the formation of new antioxidant compounds, which will increase the overall antioxidant activity (Eriksson and Na, 1995; and Nicoli *et al.*, 1997). Hence, the effect of processing on antioxidant activity of plant materials can be positive, negative or neutral (Makris and Rossiter, 2001).

For example, Gazzani *et al.* (1998) observed changes in water-soluble antioxidant properties of some vegetable juices, such as celery, eggplant, onion, tomato and zucchini. The protective activity (PA%) of frozen juices slightly decreased compared to fresh juices while freeze-dried juices increased their PA value, especially in the case of vegetables that showed low and variable PA values. This probably implied that the

oxidants, or compounds reacting with antioxidant, are susceptible to processing treatments.

Same researchers also reported that thermal treatments, e.g. boiling, caused a decrease in PA. This is in contrast with other reports where antioxidant activity *in vitro* was analyzed by chemical assay (Gazzani *et al.*, 1998). This may be because the thermal inactivation of oxidant substances, such as enzymes, apparent *in vitro*, is not revealed by biological assays where the antioxidant defense mechanisms of living being may come into play.

As stated before, anthocyanin plays a major role in antioxidant capacity of fresh Roselle extract (Tsai *et al.*, 2002). After the extract is subjected to heat processing and storage, anthocyanin level declines, but there was only relatively small decrease in antioxidant activity due to the increase of other phenolic compounds formed during the processing and storage.

Many studies on antioxidant capacity of tomato, coffee and tea affected by thermal processing have shown that a prolonged heating time increases the antioxidant capacity of these samples. This is due to the formation of Maillard's reaction products (MRPs), which are known to have antioxidant capacity (Nicoli *et al.*, 1995 and 1999). The antioxidant activities of MRPs, as chain breakers, oxygen scavengers and metal chelating agents, were reported by Lingnert and Waller (1983), and Anese *et al.* (1999a,b). Thus, the development of Maillard's reaction as a consequence of heating causes the formation of compounds with antioxidant properties. This minimizes the loss of the natural antioxidants, or even enhances the overall antioxidant properties of the samples.

The stability of pigments in dried fruits is influenced by several predrying treatments. Stability during processing can be a function of blanching temperature, water activity, pH and the presence of pro- or anti-oxidants (Von Elbe, 1987). Kearsley and Rodriguez (1981) reported that the color of anthocyanins in glycerol-water mixture increased as a_w decreased.

Skrede *et al.* (2000) reported the activity of native polyphenol oxidase in the degradation of anthocyanins during milling and in the depectinization in blueberry juice processing. Wightman and Wrolstad (1996) also reported the activity of beta-glucosidase as a factor in total monomeric anthocyanin and cyanidin-3-glucoside decreases in boysenberry juice. The experiment in cranberry juice conducted by the same group in the previous year (Wightman and Wrolstad, 1995) indicated the same tendency and also showed that the loss of the pigment was much higher when enzymes were used with juice than with crushed fruit.

Comparing the effects of different drying methods, freeze-dried samples yielded the highest content of anthocyanins, while oven drying at 105°C destroyed almost 75% of anthocyanins. The thermal decomposition of total anthocyanins in eggplant extract seemed to proceed as a first order reaction (Ishi *et al.*,1990).

Sain and Ishak (1991) reported that anthocyanin concentration decreased progressively in pineapple and papaya as blanching temperature and time increased. This could be because of the heat lability of anthocyanins as well as the leaching and dissolving of water soluble anthocyanins into blanching water (Markakis, 1974). However, blanching was found to have a protective effect on anthocyanin pigments in strawberry (Wrolstad *et al.*, 1970).

According to Wrolstad *et al.* (1970), over 90% of anthocyanin pigments were degraded after storing strawberry concentrate at 20°C for 8 weeks, but the samples were still reddish in colour. This result confirmed the experiment performed by McKinney and Chichester (1952). The latter group also suggested that the development of the products from browning reaction resulted in the immediate loss of strawberry preserves in attractiveness. Skrede *et al.* (1992) discovered that the color stability was dependent upon total anthocyanin concentration rather than qualitative pigment composition in strawberry and blackcurrant syrups.

The action of heat on some plant varieties causes pinkish discolouration attributed to the decomposition of colorless leucoanthocyanins to red anthocyanins which has been suggested to be an acid-catalyzed dehydration by oxidation (Ranganna, and Parpia, 1974). The pinkish discoloration is primarily a function of the extent of heating, which determines the intensity of the colour produced. When cooked, the heat and the acids convert the colorless leucoanthocyanin pigments to red anthocyanins (www6).

2.5 Additional studies required

Rhubarb has been shown to have medical properties, e.g. a lipid lowering effect (Abe *et al.*, 1995 and 2000; Abe and Prestwith, 1998; and Goel *et al.*, 1997, 1998 and 1999) and the ability to modulate sugar absorption (Reimer *et al.*, 1997 and 2000; and Yokozawa, 1997). Rhubarb juice has also been reported to have antioxidant properties (Son *et al.*, 2000; and Oszmianski, 1994).

However, these properties may be affected by some processing treatments applied to preserve the plant and its products. As several reports have demonstrated, the effects of

some processing treatments on the antioxidant capacity and dietary fiber fractions of plant materials can be quite unpredictable. These, in turn, would have a profound effect on their nutritional and functional properties. The effects of some processing treatments, e.g. freezing, blanching, drying and concentration on rhubarb pulp and juice have not be fully elucidated and, therefore, need to be studied.

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CHAPTER 3

Materials and Methods

3.1 Materials

Rhubarb stalks, cultivar German Wine and Macdonald, were either harvested from local growers or purchased from local market in Edmonton, Alberta. Those not used within one week were packaged in plastic bags and kept at -20 °C after being harvested, washed and cut to about 15 cm in length. Stalks to be used fresh were packaged in plastic bags, after being washed and cut, and kept at 4 °C.

Three batches of rhubarb were used throughout the project as indicated in Table 3.1. The first batch was frozen and used to prepare dried frozen rhubarb pulp. The second batch was used fresh to prepare dried fresh rhubarb pulp as well as fresh and frozen rhubarb juice. The last batch was used to determine the effects of freezing on rhubarb pulp in comparison with fresh pulp. Figure 3.1 shows the processing flowchart for dried rhubarb pulp and rhubarb juice.

Table 3.1: Batches of rhubarb used in the project

Batch	Harvesting Date	Possible Cultivar	Sample Prepared
1	Sep. 2001	Macdonald	Dried frozen rhubarb pulp
2	June, 2002	Macdonald	Dried fresh rhubarb pulp; and fresh and frozen rhubarb juice
3	August, 2002	German Wine	Dried frozen and fresh rhubarb pulp for investigating the effects of freezing

3.1.1 Materials required for oxalate removal from rhubarb juice

- Raw fresh and frozen rhubarb juices
- 1M Calcium chloride solution
    - Calcium chloride (Fisher Scientific, ON, Canada)

3.1.2 Materials required for dietary fiber determination

- Powdered dried rhubarb pulps and freeze dried rhubarb juices
- Acetone (Fisher Scientific, ON, Canada)
- Celite 545, acid washed (Megazyme, Wicklow, Ireland)
- Cleaning solution, Micro (Labcor, PQ, Canada)
- Deionized water
- Enzymes for TDF assay (Megazyme, Wicklow, Ireland)
    - Alpha-amylase, heat-stable
    - Protease
    - Amyloglucosidase
- 78% and 95% Ethanol
    - Ethanol (Commercial Alcohols, MB, Canada)
- 0.561N Hydrochloric acid
    - Conc. hydrochloric acid (Fisher Scientific, ON, Canada)
    - MES/TRIS buffer, 0.5M each, pH 8.2
        - 2(*N*-morpholino)ethanesulfonic acid (MES) (Sigma, ON, Canada)
        - Tris(hydroxymethyl)aminomethane (TRIS) (Sigma, ON, Canada)

- pH standards buffer solution at pH 4.0, 7.4 and 10.0 (Fisher Scientific, ON, Canada)

3.1.3 Materials required for total anthocyanin content determination

- Powdered dreid rhubarb pulps and rhubarb juices
- Acidified methanol: 0.01% (v/v) hydrochloric acid in methanol
- C_{18} Sep-Pak cartridge (Waters, ON, Canada)
- Deionized, distilled water
- Ethyl acetate (BDH Inc., ON, Canada)
- Conc. hydrochloric acid (Fisher Scientific, ON, Canada)
- Methanol (Fisher Scientific, ON, Canada)
- 0.025M Potassium chloride buffer, pH 1.0
    - Distilled water
    - Conc. hydrochloric acid (Fisher Scientific, ON, Canada)
    - Potassium chloride (BDH Inc., ON, Canada)
- 0.4M Sodium acetate buffer, pH 4.5
    - Distilled water
    - Conc. hydrochloric acid (Fisher Scientific, ON, Canada)
    - Sodium acetate (Fisher Scientific, ON, Canada)

3.1.4 Materials required for pH measurement

- Powdered dried rhubarb pulps and rhubarb juices
- pH standards buffer solutions at 4.0, 7.0 and 10.0 (Fisher Scientific, ON, Canada)

3.1.5 Materials required for proximate analysis

3.1.5.1 Ash content

- Powdered dried rhubarb pulps

3.1.5.2 Fat analysis

- Powdered dried rhubarb pulps and freeze dried rhubarb juices
- Petroleum ether (Fisher Scientific, ON, Canada)

3.1.5.3 Moisture content

- Powdered dried rhubarb pulps, freeze dried rhubarb juices and rhubarb juices

3.1.5.4 Protein content

- Powdered dried rhubarb pulps and freeze dried rhubarb juices
- Ethylenediaminetetraacetic acid (EDTA) (Aldrich Inc., ON, Canada)

3.1.6 Materials required for antimicrobial activity of rhubarb juices

- Powdered dried rhubarb pulps and freeze dried rhubarb juices
- Ethanol (Fisher Scientific, ON, Canada)
- Deionized water
- Distilled water
- Microbial cultures (Department of AFNS, University of Alberta, Canada)
  - *Bacillus cereus* ATCC 14579
  - *Escherichia coli* ATCC 43895
  - *Listeria monocytogenes* Scott A
  - *Salmonella typhimurium* ATCC 13311
  - *Staphylococcus aureus* ATCC 25923
- Microbial media
  - Agar, granulated (Difco, ON, Canada)
  - Tryptic Soy Broth (TSB) (Bacto, ON, Canada)
- 0.1% Tween 80 solution
  - Tween 80 (Difco, ON, Canada)

3.1.7 Materials required for bile salt binding capacity determination

- Powdered dried rhubarb pulps
- Colestid (Pharmacia Canada Inc., BC, Canada)
- 20μM Phosphate buffer, pH 7.4
  - Potassium phosphate (Fisher Scientific, ON, Canada)
  - Potassium dihydrogen phosphate (Fisher Scientific, ON, Canada)
- Scintillation liquid (ICN, CA, U.S.A.)
- Bile salt solution, 50μM each
  - Taurocholic acid (Sigma, ON, Canada)
  - [24-^{14}C]Taurocholic acid, specific activity of 2000 dpm/μmol

3.1.8 Materials required for total antioxidant capacity determination

The determination was performed by Brunswick Laboratories.

3.1.9 Materials required for water holding capacity (WHC) determination

- Powdered dried rhubarb pulps
- Distilled water

3.2 Equipment

3.2.1 Equipments required for oxalate removal from rhubarb juice

- Centrifuge, Beckman J2-21 (Beckman, ON, Canada)
- Rotary evaporator with water aspirator, 45°C, Buchi 461 (Buchi, ON, Canada)

3.2.2 Equipments required for dietary fiber determination

- Muffle furnance, 525 ± 5°C, Sybron Thermolyne F-A1730 (Sybron Thermolyne, ON, Canada)

- Oven, 103 ± 2°C and 130 ± 3°C, Despatch (Despatch, ON, Canada)
- Shaking water bath, 60 ± 1°C, Memmert (Memmert, Schwabach, Germany)
- Vacuum source

3.2.3 Equipments required for total anthocyanins content determination

- Centrifuge, 900 rmp, MSE (MSE, Alberta, Canada)
- Rotary evaporator with water aspirator, 40°C, Buchi 461 (Buchi, ON, Canada)
- Spectrophotometer, Hewlett Packard 8452A Diode Array (Hewlett Packard, ON, Canada)

3.2.4 Equipments required for pH measurement

- pH meter, Accumet 925 pH/ion Meter (Fisher Scientific, ON, Canada)

3.2.5 Equipment required for proximate analysis

3.2.5.1 Ash content analysis

No equipment required

3.2.5.2 Fat analysis

- Goldfisch unit (Labconco, ON, Canada)

3.2.5.3 Moisture content

- Oven, 103 ± 2°C, Despatch (Despatch, ON, Canada)

3.2.5.4 Protein content

- Nitrogen determinator, Leco FP-428 (Leco Co., MI, U.S.A.)

3.2.6 Equipments required for antimicrobial activity determination

- Autoclave (AMSCO, ON, Canada)
- Safety cabinet, Labconco purifier class II safety cabinet: 36204-04 (Labconco, ON, Canada)

- Safety cabinet, Model: BM6-2A-49 (Canadian Cabinets Co.Ltd., ON, Canada)
- Centrifuge, 9000 rmp, Beckman microfuge II (Beckman, ON, Canada)
- Centrifuge, 9000 rmp, MSE (MSE, ON, Canada)
- Incubator, 37.0 ± 0.2°C, Hotpack incubator multi-mode control system (Andor Scientific Services Ltd., ON, Canada)
- Rotary evaporator with water aspirator, 45°C, Buchi 461 (Buchi, ON, Canada)
- Shaking water bath, 60 ± 1°C, Memmert (Memmert, Schwabach, Germany)

3.2.7 Equipments required for bile salt binding capacity determination

- Liquid Scintillation Counter, LS 3801 Beckman coulter (Beckman, ON, Canada)
- Shaking water bath, 37.0 ± 0.2°C (Bellco Biotechnology, NJ, U.S.A.)

3.2.8 Equipments required for total antioxidant capacity determination

The determination was performed by Brunswick Laboratories.

3.2.9 Equipments required in Water Holding Capacity (WHC) determination

- Centrifuge, 9000 rmp, MSE (MSE, ON, Canada)
- Shaking water bath, 60 ± 1°C, Memmert (Memmert, Schwabach, Germany)

3.3 Processing

3.3.1 Rhubarb Pulp and Juice Preparation

For the experiments with fresh stalks, juice was separated from the stalks within two days of storage in walk-in cooler. For the experiments with frozen stalks, the stalks were left to thaw at room temperature until they reached 20-24 °C before further processing. As illustrated in Figure 3.1, the stalks, either fresh or thawed, were cut into

pieces of 1 cm in length, and pressed with a manual screw press to separate the juice. The pressed pulp was either steam-blanched for 10 min before drying (1), or dried directly (2) in either of the three types of dryers for comparison:

1. Freeze dryer (The VirTis Co., Inc., NY, U.S.A.)
   - Drying conditions: 500 mTorr and condenser temperature = -60°C
2. Fluidized bed dryer (North American Rockwell, MA, U.S.A.)
   - Drying conditions: 85°C, 2 h
3. Drum dryer (Reliance Electric Co., OH, U.S.A.). The drying was done in two stages:
   i. Pre-drying in drum dryer at pressure 20 psi., drum speed 1 and the clearance between drums was 1 mm
   ii. Drying in air dryer at 75 °C for 2 h to complete the drying process

The dried pulps were ground finely in a coffee grinder. The ground fiber was sifted through a 60 mesh sieve, vacuum packaged in polyethylene bags and stored at - 20°C.

3.3.2 Removal of oxalate from Juice

Oxalic acid in rhubarb juice, extracted from either frozen or fresh rhubarb stalks, was removed according to Cai *et al.* (2000), using calcium chloride treatment by adding 7 mL of 1 M calcium chloride solution to 100 mL rhubarb juice. The juice was then centrifuged at 1912xg (3000 rpm). The supernatant was concentrated using vacuum evaporator at 45°C to 50%, 65% and 75% total soluble solid (TSS) and stored at –20 °C.

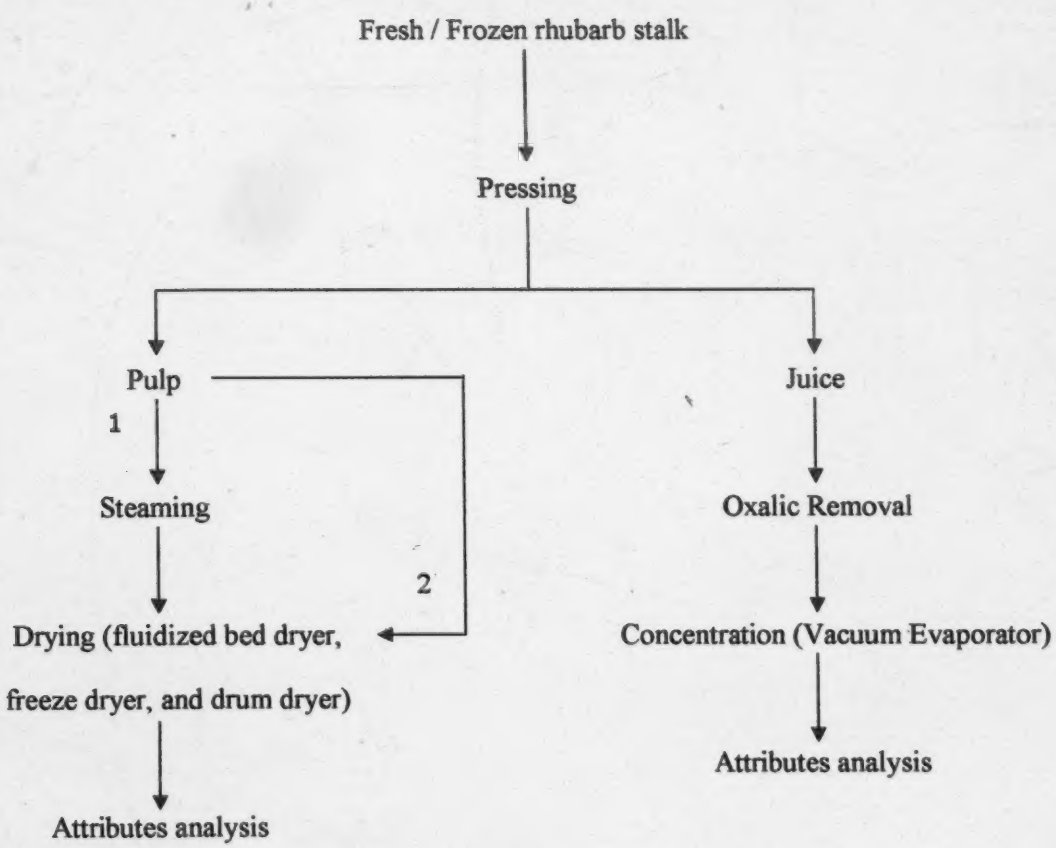

Figure 3.1: Rhubarb pulp and juice preparation

3.4 Chemical analysis

3.4.1 Determination of dietary fiber

The insoluble, soluble and total dietary fiber contents of rhubarb pulps and juices were determined by enzymatic methods described in AOAC Method 991.43.

3.4.2 Determination of total anthocyanin content

3.4.2.1 Sample preparation

The sample extraction and anthocyanin purification were performed using the method described by Wrolstad (1976). Briefly, 10 mL acidified methanol was mixed with 5 g of pulp samples and allowed to macerate for 1 h at room temperature. The mixture was then filtered through a Whatman No.1 filter paper using vacuum suction. The samples were re-extracted with acidified methanol until a faint-colored extract was obtained, and the filtrates combined.

The concentrated rhubarb juice samples were diluted to 10% TSS before the purification of anthocyanin, using a C18 cartridge. The raw juice samples were used at their original concentration. All data obtained, in case of rhubarb juice, were calculated to show the total anthocyanin contents at 3% TSS for comparison.

The aqueous anthocyanin extract was pumped through a C18 cartridge (C18 Sep-Pak, Waters Chromatography), the cartridge was washed with two column volumes of acidified water and ethyl acetate, then anthocyanin pigments were eluted with acidified methanol. The aqueous eluants were evaporated in vacuum evaporator at 40 °C to remove methanol, then re-dissolved in acidified deionized distilled water to a known volume and stored at -20 °C.

3.4.2.2 Measurement of total anthocyanin content

The measurement was performed using the pH differential method described by Somers and Evens, (1974) and Wrolstad *et al.* (1982). Briefly, the anthocyanin extracts were mixed with potassium chloride buffer (pH 1.0) using the proportions which would give the absorbance of the sample at $\lambda_{vis\text{-}max}$ within a linear range. The extracts were also

mixed with sodium acetate buffer (pH 4.5), using the same ratios. The absorbance of the solutions were measured at the $\lambda_{vis\text{-}max}$ and at $\lambda_{700\ nm}$. The absorbance was calculated using the following equation:

$$A = (A_{\lambda vis\text{-}max} - A_{700})_{pH\ 1.0} - (A_{\lambda vis\text{-}max} - A_{700})_{pH\ 4.5}$$

The monomeric anthocyanin pigment concentrations in the original samples were obtained using the following formula:

Monomeric anthocyanin pigment (mg/L) = (A x MW x DF x 1000)/(ε x 1)

Where MW is the average molecular weight of cyaniding-3-glucoside and cyaniding-3-rutinoside (520), DF is the dilution factor, and ε is the molecular absorptivity of cyaniding-3-glucoside and cyaniding-3-rutinoside (29200).

3.4.3 pH measurement

pH measurement of rhubarb pulps and juices was conducted accorded to the AOAC Method 943.02.

3.4.4 Proximate analysis

Ash contents of rhubarb pulps and juices were obtained during the determination of dietary fiber. The fat and moisture contents were determined using AOAC Method 920.125 and Method 984.25, respectively. Protein contents were measured by FP-428 Nitrogen Determinator (LECO Instruments, Mississauga, ON, Canada) using nitrogen factor = 6.38.

3.5 Nutritional studies

3.5.1 Determination of antimicrobial activity

3.5.1.1 Sample preparation

The rhubarb pulp extracts were obtained from the following two methods:

1. Rhubarb samples (10 g) were extracted with an equal amount of 95% ethanol (w/v) in a water bath at 50°C for 1h. The mixtures were filtered through Whatman No.1 filter paper using vacuum filter. The residues were re-extracted with the same amount of ethanol and then filtered. The combined filtrate was concentrated in a rotary evaporator at 45°C to dryness, and made up to 10 mL with 0.1% Tween 80 aqueous solution.

2. Rhubarb samples (10 g) were blended with an equal amount of deionized water in a blender. After the homogenization, the mixtures were filtered through Whatman No.1 filter paper using vacuum filter, then concentrated on a rotary evaporator at 45°C, and adjusted to the volume of 10 mL with 0.1% Tween 80 aqueous solution.

The extracts obtained from the two methods were centrifuged at 11,200 g for 10 min to precipitate suspended particles and the supernatants were used as stock solutions for antimicrobial tests (Pao-Chuan et al., 2001).

Rhubarb juices and rhubarb juice concentrates were used at their actual concentrations, and the concentrates with 65% and 75% TSS were also diluted to 50% TSS to investigate the effect of concentration time.

According to Cai *et al.* (2000), oxalic, malic and citric acids are the major organic acids present in rhubarb and are responsible for its low pH. The three acids are present in rhubarb at the ratio of 0.45:5.75:0.71 (g:g:g) in 100 mL juice. Because oxalic acid was

removed completely from the juice during the juice preparation step, only malic and citric acids remained and were responsible for the low pH of rhubarb juice. Thus, the solutions of malic and citric acid mixed at the ratio of 5.75:0.71 (g:g) and pH adjusted to 0.9, 1.0, 1.2, 1.5, 2.0, 2.5 and 3.2 were also used in the antimicrobial experiment to observed the effect of the pH *per se*.

3.5.1.2 Preparation of test microorganisms

Microorganisms used were *Bacillus subtilis*, *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Salmonella typhimurium* and *Staphylococcus aureus*. All test microorganisms were grown on Tryptic Soy Agar (TSA) plates. The test strains were transferred to fresh Tryptic Soy Broth (TSB) and their absorbance at 600 nm adjusted to 0.1 before use.

3.5.1.3 Antimicrobial Assay

The determination of antimicrobial activity was performed using the Paper Disk Method. Briefly, a 0.1 mL TSB contained cultures were spread on agar plates using a glass rod spreader. The plates were left at room temperature for 20 min to allow the surface to dry. Sterilized filter paper disks (Whatman No.1 filter paper, 6 mm in diameter) were arranged on the plate and a 10 µL aliquot of test solutions were added to the paper. Seven disks were placed on a plate, six for the different extracts and one for the control. Distilled water was used as the control. All plates were incubated at 37°C for 12, 18, 24 and 48 h. The diameter of the distinctly clear zones were measured and expressed in millimeters (Jeongmok *et al.*, 1995). Two disks were used for each test solution.

3.5.2 Determination of bile salt binding capacity

The determination of *in vitro* bile salt binding capacity was performed using the method described by Goel *et al.* (1998). Briefly, 100 mg rhubarb pulp samples and colestipol (Colestid, Upjohn Pharmaceuticals) were incubated with 10 mM [24-^{14}C] taurocholic acid (specific activity of 2000 dpm/μmol) in 5 mL phosphate buffer (pH 7.4) at 37°C for 1 h, and then the incubated mixtures were filtered through a filter paper with 45 μm pore size. A 0.5 mL sample of the supernatants were counted for radioactivity in Liquid Scintillation Counter. The amount of bile salt bound was calculated as the difference between the amount of bile salt added and that recovered in the supernatant.

3.5.3 Determination of total antioxidant capacity

3.5.3.1 Sample preparation

The preparations of dried rhubarb pulps for the analysis was done by Brunswick Laboratories (Wareham, MA, U.S.A.). The juice samples were diluted to 3% TSS before being used in the experiment.

3.5.3.2 Total antioxidant assay

Unlike other nutrients, antioxidants are chemically diverse. The most common antioxidants present in vegetables are vitamin C and E, carotenoids, flavonoids, and thiol (SH) compounds, etc. The chemical diversity of antioxidants makes it difficult to separate and quantify individual antioxidants from the plant matrix, but it is possible to determine the total antioxidant activity directly from plant extracts.

Several methods have been developed to measure total antioxidant activity (Ou *et al.*, 2002). For example, Trolox Equivalent Antioxidant Capacity (TEAC), Ferric Reducing Antioxidant Power (FRAP), and Oxygen Radical Absorption Capacity (ORAC) assays are the representative ones.

These methods are based on either single electron transfer (SET) reaction or a hydrogen atom transfer (HAT) reaction between an oxidant and a free radical.

The ORAC and FRAP values of vegetables are not only dependent on species, but also greatly depend on cultivation conditions and harvest time. Besides, the two assays give different antioxidant activity trends. The experiment conducted by Ou *et al.* (2002) indicated that the ORAC assay is chemically more relevant to chain-breaking antioxidants activity, while the FRAP method has some drawbacks, such as reaction kinetics and quantitation methods.

Since the ORAC assay directly measures the antioxidant capacities of chain-breaking antioxidants against peroxyl radicals, it can probably be used as a guideline for "peroxyl radical absorption capacity" of plants.

The total antioxidant assay used in this experiment, i.e. ORAC, was created by Guohua *at al*, (1993) and improved by Ou *et al.* (2002). Briefly, the fluorescence of a 3 mL mixture of 1.67 x 10^{-8} M fluorescein, 3 x 10^{-3} M 2,2'-azobis(2-amidinopropane) dinydrochloride (AAPH), and 30 μL sample extract in 10^{-2} M phosphate buffer (pH 7.0) was measured every 5 min at the emission of 565 nm and excitation of 540 nm using fluorescence spectrophotometer until zero fluorescence occurred. Trolox solution was used during each run as a standard.

AAPH is used as a peroxyl radical generator in which its presence will change the fluorescence intensity of a fluorescent probe, fluorescein. The ORAC assay depends on the free radical damage to this fluorescent probe. The change of fluorescence intensity is an index of the degree of free radical damage. In the presence of antioxidants, the inhibition of free radical damage by an antioxidant, which is reflected in the protection against the change of probe fluorescence in the ORAC assay, is the measure of its antioxidant capacity against the free radical. The uniqueness of this assay is that the reaction is driven to completion and the quantitation is achieved using 'area under the curve' plotted between the fluorescence intensity and time (Brunswick Laboratories, 2003).

3.6 Water holding capacity (WHC) determination

WHC of the pulp samples were determined according to the method of Morrow and Lorenz (1974). A 0.5 g of pulp samples were mixed with 10 mL distilled water. The mixtures were placed in a shaking water bath (at 24 °C, speed 5) for 1 h., then centrifuged for 30 min at 2000xg. The supernatants were discarded. The liquid retained by the solids was determined by the difference in sample weights before and after hydration. WHC is expressed as grams of water retained per gram of dry sample.

3.7 Statistical analysis

Statistically significant differences among means were evaluated with ANOVA (one way analysis of variance). Means were considered to be significantly different at $P < 0.05$.

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CHAPTER 4

Results and Discussion

Rhubarb is composed principally of pulp and juice. Rhubarb pulp has been shown to possess some desirable functional and nutritional properties, e.g. high dietary fiber content, high water holding capacity (WHC), high bile salt binding capacity (BSBC), can reduce cholesterols and triglycerides, and can modulate sugar absorption (Ooraikul, *et al.*, 1993 and Goel, *et al.*, 1999). It can also be used as a texturizing ingredient in products such as chicken surimi (Attapatu, 1993), vegetarian products (Shum, 1998), and high-fiber meat products (Ooraikul *et al.*, 1993). Rhubarb juice has been shown to have high concentration of pectin, and acidity, suitable for use as an acidulant (Cai *et al.*, 2000) and for making products such as jam, jelly and topping (Ooraikul *et al.*, 1993).

It is very likely, especially with highly pigmented rhubarb cultivars, that both pulp and juice contain significant amounts of antioxidant compounds, especially phenols or anthocyanins, thus may be effective as antioxidant products. Furthermore, due to high acidity and phenolic compounds, rhubarb juice may also possess antimicrobial property. However, these properties have not been clearly demonstrated.

In order to produce rhubarb pulp and juice, rhubarb stalks have to undergo several processing treatments, some of which may have significant effects on the properties of these products. Besides cleaning, trimming and cutting, these processing treatments include cooling (for fresh use) or freezing (for long-term storage), and may be blanching before pressing to inactivate deteriorative enzyme systems. Blanching can also cause thermal hydrolysis of pectins and fiber. For pulp, it may subsequently be dried and

pulverized if it is to be used as a bulking agent in food products, or a nutritional ingredient in nutraceuticals or functional foods. For juice, it may be frozen if it is to be used as a single-strength fresh juice, or it may be thermally concentrated to reduce volume for long-term storage and use as an ingredient for jam, jelly, topping, etc.

This research was designed to address this gap in knowledge regarding rhubarb products, and to elucidate the effects of some processing treatments on composition and functional properties of these products. The following are the results from this research.

4.1 Effects of freezing on rhubarb pulp

For long-term storage and to extend processing season right through the year, rhubarb stalks are generally frozen. In this study, rhubarb stalks were cleaned, trimmed and cut into short pieces before packing in plastic bags and frozen in the Department's walk-in freezer. Therefore, the freezing rate was slow, producing large ice crystals, which likely created significant structural damage to rhubarb tissue. This was clearly apparent, since rhubarb pieces became soft after thawing, with juice oozing out. Thus, the juice could be more easily pressed out from the pulp as compared with that from fresh rhubarb.

Freezing rate is the main factor controlling size and location of ice crystals formed during the freezing period. Fast freezing leads to the simultaneous formation of many ice crystals immediately as temperature quickly drops below the freezing point, leading to the formation of many small ice crystals, both within the cells and in the intercellular space. On the other hand, if the liquid is cooled slowly, the ice crystals will be slowly formed, allowing water to permeate from the cells toward the crystals making them grow larger. However, less crystals will be formed under these conditions as

compared to those under rapid freezing (Brown, 1979). Most of ice crystals formed during slow freezing are located in the space between cells.

The changes of plant's compositions during freezing were reported by several researchers. The effects of freezing on plant tissues depend on the type of vegetable as well as storage conditions. For example, the vitamin C content in strawberries changed slightly during storage at -20°C for 6 months, but the anthocyanin level was reduced to less than half after the same storage period (Hong and Ueda, 1993). The parallel decrease of total anthocyanins and total antioxidant capacity was observed during storage of onions mimicking home-style storage for 6 weeks (Gennaro *et al.*, 2002), while refrigeration of red radish and red-fleshed potato juices at 2°C prolonged the anthocyanin half-life to over a year (Rodriguez-Saona *et al.*, 1999). Svanberg *et al.* (1995) reported a decrease of soluble fiber content and increase of insoluble fiber in carrots under similar storage conditions.

The composition, functional and nutritional properties of rhubarb pulp produced from frozen stalks as compared to that from fresh stalks were analyzed and the following are the results.

4.1.1 Effects of freezing on chemical composition

The results in Table 4.1 showed that freezing rhubarb stalks before pressing out the juice led to significant losses in soluble dietary fiber, total anthocyanins, protein and fat contents, while the levels of insoluble dietary fiber and ash contents remained unchanged.

Table 4.1: Chemical composition of fresh and frozen rhubarb pulps (dry weight basis)

Attributes	FsRP[1]	FzRP[2]
Insoluble dietary fiber (%)	59.97 ± 0.09^a	60.06 ± 0.11^a
Soluble dietary fiber (%)	11.78 ± 0.05^a	10.51 ± 0.13^b
Total dietary fiber (%)	71.75 ± 0.04^a	70.56 ± 0.01^a
TACN[3] (mg/L)	6.49 ± 0.02^a	5.92 ± 0.06^b
Protein content (%)	16.45 ± 0.06^a	15.38 ± 0.06^b
Fat content (%)	0.89 ± 0.00^a	0.87 ± 0.00^b
Ash content (%)	9.80 ± 0.08^a	9.80 ± 0.04^a

Values are means $\pm$ SD. Values, presented in the same row, not sharing a common superscript letter are significantly different at $p < 0.05$.
[1] FsRP = Fresh rhubarb pulps; and [2] FzRP = Frozen rhubarb pulps
[3] TACN = Total anthocyanin (1 L of sample was extracted from 500 g of dried rhubarb pulp)

The decrease of anthocyanins and soluble dietary fiber contents in frozen sample could be attributed to cell damage resulting in drip loss of rhubarb juice (Meryman, 1971 and Steponkus, 1984). Mohr (1971) reported the alteration of rhubarb stalk after a freeze-thaw process. A considerable leakage of juice and flabby texture after thawing were observed. These changes are probably the result of damage to protoplasmic membranes resulting in the loss in cells' liquid and turgor pressure. Water and soluble substances may leak out through the cut surfaces and damaged cell walls (Brown, 1979).

The cell shrinkage during freezing made it easier to separate the juice by pressing, giving a greater yield of juice as compared to that obtained from pressing fresh stalks.

Though low temperature decreases the rate of reaction, non-enzymatic reactions may occur faster as the formation of ice increases the concentration of the reactants in the unfrozen liquid (Brown, 1979). Activities of some enzyme systems were observed at extreme conditions. For example, pectin methyl esterase (PME) was reported to demethoxylate pectin when the tissue was damaged by heating or freezing (Van Buren,

1979). The disruption of the normal compartmentalization of living cells also helps accelerate the undesirable chemical reactions during freezing. De-esterification of pectin was also observed in cherries during storage at -6.7°C by Guadagni *et al.* (1958) and Gee and McCready, (1957).

Anthocyanins are largely responsible for the red or purple color of rhubarb stalks. It should be noted that rhubarb stalks used in this freezing experiment were largely greenish in color while those used in the blanching experiment (Section 4.2.1, Table 4.3) were mainly red or purple. The former was most likely of German Wine cultivar, while the latter could be of Macdonald cultivar. Both were obtained from local growers at different times, depending on their availability, and the identification of the cultivars was uncertain. This resulted in a substantial discrepancy in the total anthocyanin contents of the pulp samples shown in Table 4.1 and Table 4.3. Note that the concentrations of other components of the samples in both tables are essentially similar, therefore, it is not likely that analytical errors caused this discrepancy.

4.1.2 Effect of freezing on functional and nutritional properties

The ability to bind bile salt and trap water of rhubarb pulp from frozen stalks decreased significantly compared to that of rhubarb pulp from fresh stalks (Table 4.2). This could be due to the decrease in soluble dietary fiber (Table 4.1) whose major component is pectins. Pectins and hemicelluloses were reported to have a high water holding capacity (WHC) (Schneeman, 1986).

Table 4.2: Nutritional and functional properties of fresh and frozen rhubarb pulps (dry weight basis)

Attributes	FsRP[1]	FzRP[2]
BSBC[3]	12.26 ± 0.62^{a}	10.81 ± 1.07^{b}
Water holding capacity (g/g)	12.11 ± 0.30^{a}	11.13 ± 0.14^{b}

Values are means $\pm$ SD. Values, presented in the same row, not sharing a common superscript letter are significantly different at $p < 0.05$.
[1] FsRP = Fresh rhubarb pulps; [2] FzRP = Frozen rhubarb pulps; and [3] BSBC = Bile salt binding capacity (μmole Taurocholate/100mg dried fiber)

According to Robertson and Eastwood (1981), WHC was largely affected by tissue structure alteration, which was observed in frozen rhubarb stalk by Mohr (1971) as well. Brown (1979) attributed the texture change of plant tissue during freezing to the loss of turgor, or internal pressure, and the breakage of cell walls by ice crystals especially in slow freezing leading to the loss of crispness. In damaged plant tissues, the size of ice crystals possibly is larger than the size of a single cell. During slow freezing, an individual ice crystal may grow through cell wall pores from one cell to another, resulting in higher damage of cells structure.

4.2 Effects of blanching on rhubarb pulp

The objective of the blanching step in the production of dried rhubarb pulp is to soften rhubarb stalk tissue and inactivate deteriorative enzymes prior to further processing. The two most widespread blanching methods used are steam and hot water blanching. Steam blanching, which, compared to hot water blanching, causes lower loss of water-soluble components, was used in the preparation of dried rhubarb pulp samples in this experiment.

Heat treatment such as blanching can cause damage in cell barrier. This damage changes cell wall characteristics, rendering it unable to support the osmotic process, so molecules of solutes can translocate through it more easily. Jewell (1979) reported that steam blanching caused protoplasmic degradation and formation of numerous vesicles, but cell wall degradation under steam blanching was lower compared to water-blanched samples.

Nyman *et al.* (1987) observed the breakage of glycosidic linkages in the dietary fiber polysaccharides during heat treatment. The alteration in dietary fiber fractions depends on the intensity of heat applied. For example, under a moderate condition, the amount of soluble fiber will increase, and there will be an apparent decrease in the total DF content if subjected to severe heat treatment.

The composition, functional and nutritional properties of dried rhubarb pulp, with and without blanching, were compared in the following sections.

4.2.1 Effect of blanching on chemical composition

Blanching process caused the decrease of insoluble dietary fiber, protein and ash contents in rhubarb pulp samples as shown in Table 4.3. On the other hand, the process increased soluble dietary fiber and total anthocyanin levels while the fat level remained unchanged. Antioxidant capacity of the blanched samples also increased (Table 4.6).

Blanching process leads to the softening of plant tissues. This is primarily due to the disruption of cell walls and the degradation and solubilisation of pectin present in the tissue.

Pectic polysaccharides are particularly susceptible to degradation. Heat treatment and the presence of acid or alkali usually lead to the increase of fiber solubility due to the

degradation of pectic polysaccharides within the fiber matrix which contains cross-linked pectic polysaccharides, hemicelluloses, cellulose, protein and lignin (Van Buren, 1979). Hydrolysis of glycosidic bond between sugar side chains or in the main chain at rhamnose units occurs when pectic polysaccharide is subjected to heat with the presence of acid resulting in the increase of its solubility.

Table 4.3: Chemical composition of dried fresh rhubarb pulps (dry weight basis)

Attributes	FDRP[1]	
	Un-blanched	Blanched
Insoluble dietary fiber (%)	62.34 ± 0.99^{a}	58.08 ± 0.10^{b}
Soluble dietary fiber (%)	11.08 ± 0.86^{b}	13.50 ± 0.18^{a}
Total dietary fiber (%)	73.42 ± 0.13^{a}	71.58 ± 0.08^{a}
TACN[2] (mg/L)	505.09 ± 9.31^{b}	519.06 ± 8.88^{a}
Protein content (%)	10.90 ± 0.01^{a}	10.55 ± 0.08^{b}
Fat content (%)	0.78 ± 0.01^{a}	0.77 ± 0.00^{a}
Ash content (%)	10.04 ± 0.29^{a}	9.95 ± 0.11^{a}

Values are means $\pm$ SD. Values, presented in the same row, not sharing a common superscript letter are significantly different at $p < 0.05$.
[1] FDRP = Freeze-dried rhubarb pulp
[2] TACN = Total anthocyanin (1 L of sample was extracted from 500 g of dried rhubarb pulp)

The degree of depolymerization depends on several factors, such as temperature and time of the treatment (Albersheim *et al.*, 1960). According to Svanberg *et al.* (1995) if the breakdown of glycosidic linkages occurred moderately, the amount of soluble dietary fiber will increase which assumed happened here.

Gooneratne *et al.* (1994) suggested that the increase in soluble fiber content in extruded mung bean flour as compared to raw flour was mainly due to the increase in the solubility of arabinose-containing pectic polysaccharides and hemicelluloses present as

insoluble-fiber in the raw flour. The solubilisation of these two compounds was also found in extruded cooked pea hulls (Ralet *et al.*,1993).

Blanching increased the anthocyanin level by inactivating the enzyme systems responsible for its degradation. According to Wrolstad *et al.*(1980), several enzyme systems were claimed to participate in the anthocyanin destruction. For example, rhamnosidase and peroxidase, as well as glycosidase can hydrolyze the glycosidic bond of anthocyanin to yield anthocyanidins (Peng and Markakis, 1963; Wightmand and Wrolstad, 1996 and Cash *et al.*, 1976). Also, amount of anthocyanins can be increased by the conversion of colorless leucoanthocyanin pigments, whose presence in rhubarb was reported by Racz *et al.* (1959), when the plant was exposed to heat and acids (www1). The mechanism of the conversion of leucoanthocyanin to anthocyanin is not yet fully understood.

4.2.2 Effect of blanching on functional and nutritional properties

The application of heat treatment leads to the increase in the solubility of dietary fiber by depolymerization of insoluble dietary fiber (Svanberg *et al.*, 1995), The chain cleavage by enzymatic or non-enzymatic reactions also results in the decrease in the molecular weight of soluble polymers (Van Buren, 1979). This depolymerization of readily soluble fiber of high molecular weight leads to the reduction in its viscosity.

The depolymerization of fiber polysaccharides is quite important from the nutritional standpoint. This is because the fiber's ability to bind bile salts and retain water also depends on the viscosity of the fiber in the solution, apart form the type of fiber and its structure (Mongeau and Brassard, 1982; Robertson and Eastwood, 1981). Since the

viscosity of the fiber solution is reduced by the cleavage of a few glycosidic linkages (Albersheim *et al.*, 1960), heat treatment of the pulp may also reduce the fiber's bile salt binding capacity (BSBC) and water holding capacity (WHC).

Further more, the degradation and solubilisation of noncellulosic polysaccharides, i.e., pectic polysaccharides and hemicelluloses, will alter the porosity of the fiber matrix (Gooneratne *et al.*, 1994), which can also lead to the deterioration of the functional properties of rhubarb pulps (Table 4.4).

Table 4.4: Nutritional and functional properties of dried fresh rhubarb pulps (dry weight basis)

Attributes	FDRP[1]	
	Un-blanched	Blanched
BSBC[2]	19.38 ± 0.63^{a}	18.34 ± 1.29^{a}
Water holding capacity (g/g)	16.83 ± 0.06^{a}	12.67 ± 0.34^{b}

Values are means $\pm$ SD. Values, presented in the same row, not sharing a common superscript letter are significantly different at $p < 0.05$.
[1] FDRP = Freeze-dried rhubarb pulp; and [2] BSBC = Bile salt binding capacity (µmole Taurocholate/100mg fiber)

Robertson and Eastwood (1981) reported that the steam pretreatment caused the compression of cells in bran samples. This alteration in bran structure can cause the lowering in its WHC. Mohr (1971) noticed changes in rhubarb associated with freeze-thaw and blanching. A gradual breakdown in the organization of the protoplasmic structure and, in most cases, the rupture of plasmalemma, causes subsequent loss of turgor pressure. Some degradation and separation of cell walls were also observed in a number of experiments (Mohr, 1974; and Monzini, 1974).

The increase of anthocyanins level, which was reported by numerous researchers to exhibit antioxidant capacity (Kalt *et al.*, 1999; Prior *et al.*, 1998 and Wada and Ou,

2002), due to blanching, could be the factor in the increase in total antioxidant capacity (ORAC) in the blanched frozen rhubarb sample (Table 4.6). A possible formation of Maillard's reaction products (MRPs), which exhibit an antioxidant capacity, can contribute to the increase of total antioxidant capacity of the blanched dehydrated pulp as well (Nicoli *et al.*, 1997).

4.3. Effects of drying on rhubarb pulp

The main purpose of drying food stuff is to extend their shelf life by the reduction of their water activity. The decrease in weight and volume also aids the transportation and lowers the storage cost.

Drying (or dehydration) of rhubarb pulps in this project involved three different methods, i.e., freeze drying, air drying and drum drying. In the first method water is removed by sublimation, while in last two drying methods, water is removed by evaporation.

Several changes are caused by dehydration process. The removal of water leads to the concentration of chemical compounds, such as proteins, carbohydrates and minerals along with some chemical changes, e.g. the oxidation of fat, leading to odor and flavor losses, Maillard's reaction involves amino compounds and reducing sugars, resulting in the darker color and new aromas in the products. Dehydration may also cause the degradation of vitamins as well as the losses of original volatile aroma and flavor compounds.

The composition and functional properties of pulp produced from frozen stalks as compared to that from fresh stalks were analyzed and the following are the results.

4.3.1 Effects of drying on chemical composition

Dietary fiber, total anthocyanins, and protein contents in aired-dried and drum-dried rhubarb pulps, both fresh and frozen, are lower as compared to those in the freeze-dried sample (Table 4.5; Fig. 4.1). Fat levels in all samples seem to be constant regardless of any processing treatments involved, as do ash contents, which appear essentially constant except that the blanched samples are slightly lower in all cases (Table 4.5).

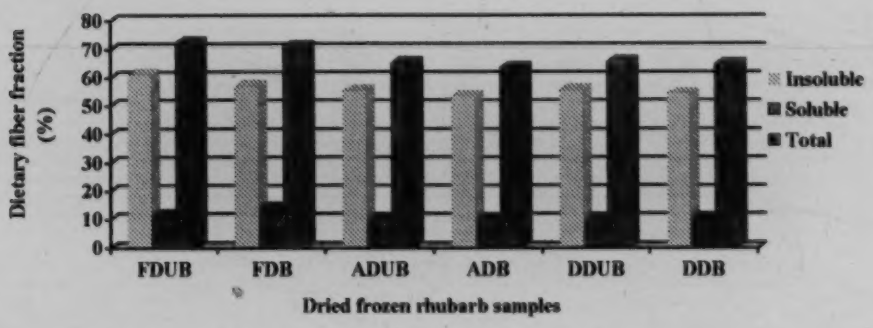

Figure 4.1: Insoluble, soluble and total dietary fiber contents in dried frozen rhubarb sample
FDUB = Freeze-dried un-blanched; FDB = Freeze-dried blanched; ADUB = Air-dried un-blanched; ADB = Air-dried blanched; DDUB = Drum-dried un-blanched; and DDB = Drum-dried blanched

Camire and Flint (1991) observed the lower of total dietary fiber in baked corn meal compared to raw cornmeal. Fornal *et al.* (1987) suggested that the fiber loss was due to dextrinization or other type of thermal decomposition.

Table 4.5: Chemical composition of dried frozen rhubarb pulps (dry weight basis)

Attributes	FDRP[1]		ADRP[2]		DDRP[3]	
	Un-blanched	Blanched	Un-blanched	Blanched	Un-blanched	Blanched
Insoluble dietary fiber (%)	60.93 ± 1.12^{a}	56.93 ± 1.29^{b}	55.37 ± 1.76^{cd}	53.29 ± 1.89^{d}	55.54 ± 0.45^{cd}	54.19 ± 0.02^{cd}
Soluble dietary fiber (%)	11.53 ± 0.35^{b}	14.11 ± 0.01^{a}	9.85 ± 0.02^{c}	10.26 ± 0.29^{c}	10.07 ± 0.72^{c}	10.46 ± 0.33^{c}
Total dietary fiber (%)	72.46 ± 1.57^{a}	71.05 ± 1.28^{a}	65.22 ± 1.74^{b}	63.55 ± 1.60^{b}	65.61 ± 0.27^{b}	64.66 ± 0.35^{b}
TACN[4] (mg/L)	604.17 ± 3.56^{b}	633.28 ± 17.09^{a}	502.84 ± 31.62^{d}	530.83 ± 8.12^{c}	371.31 ± 3.69^{f}	386.00 ± 12.27^{f}
Protein content (%)	8.46 ± 0.14^{a}	8.06 ± 0.01^{b}	7.84 ± 0.12^{c}	7.49 ± 0.14^{d}	7.82 ± 0.06^{c}	7.70 ± 0.06^{c}
Fat content (%)	0.78 ± 0.00^{a}	0.77 ± 0.05^{a}	0.80 ± 0.04^{a}	0.80 ± 0.02^{a}	0.78 ± 0.01^{a}	0.78 ± 0.04^{a}
Ash content (%)	10.05 ± 0.17^{a}	9.82 ± 0.12^{b}	9.64 ± 0.10^{bc}	9.62 ± 0.01^{c}	9.61 ± 0.05^{c}	9.60 ± 0.06^{c}

Values are means $\pm$ SD. Values, presented in the same row, not sharing a common superscript letter are significantly different at $p < 0.05$.
[1] FDRP = Freeze-dried rhubarb pulp; [2] ADRP = Aired dried rhubarb pulp; and [3] DDRP = Drum dried rhubarb pulp
[4] TACN = Total anthocyanin (1 L of sample was extracted from 500 g of dried rhubarb pulp)

During the drying process, fat can be degraded via oxidation (Colins and Maramgoni, 1998). However fat content of rhubarb samples, which is quite low compared to other components, remained stable after blanching and drying processes. This may be because most lipids present in rhubarb samples are in saturated form. Saturated fatty acids as well as their products are relatively stable (Nawar, 1985). Crnjar *et al.* (1981) reported that saturated lipids produced oxidation products when heated at temperature higher than 150°C with the presence of oxygen, while unsaturated lipids are more susceptible to oxidation at high temperature and autoxidation at low temperature.

The reduction of anthocyanins in the thermal drying treatments as compared to that in the freeze-dried sample (Table 4.5, Fig. 4.2) could be attributed to the exposure of the samples to heat. At least two degradation pathways of anthocyanins were introduced (Markakis, 1974) (Appendix 1). Markakis (1974) reported that the rate of anthocyanin degradation increases with rising temperatures. The presence of oxygen and the oxidation of ascorbic acid accelerate the degradation process (Nebesky *et al*, 1969; and Sondheimer and Kertesz, 1953). Sondheimer and Kertesz (1953) observed the parallel losses of ascorbic acid and anthocyanins in strawberry juice during storage at 30°C after subjecting to heat. This can be attributed to the production of hydrogen peroxide formed during ascorbic acid oxidation.

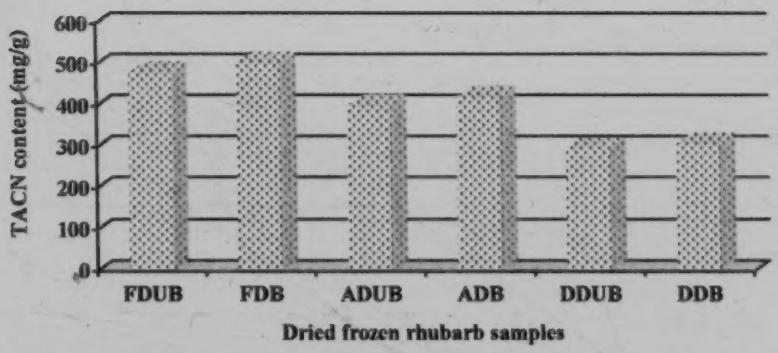

Figure 4.2: Total anthocyanin contents in dried frozen rhubarb sample
TACN = Total anthocyanin; FDUB = Freeze-dried un-blanched; FDB = Freeze-dried blanched; ADUB = Air-dried un-blanched; ADB = Air-dried blanched; DDUB = Drum-dried un-blanched; and DDB = Drum-dried blanched

Air drying seems to be able to maintain more anthocyanins in the samples as compared to drum drying. This may be due to the difference in drying temperatures of the two methods, and in sample's moisture content during the exposure to heat. In air drying, the sample was dried in fluidized bed dryer for 2 h at 85°C, and its moisture content decreased to 2-3%. In drum drying, the sample was first pre-dried by drum dryer (20 psi., 109°C) to partially lower its moisture content before continuing the drying process in a bin dryer at 75°C for 2 h until its moisture dropped to 2-3%.

The fact that, at the lower moisture content, most reactions, such as oxidation and browning, usually occur faster than those occurred at higher moisture content (Fellows, 1988) can possibly explain the higher loss in anthocyanin content in drum-dried samples as compared to air-dried rhubarb pulp. Higher drying temperature also accelerates the degradation.

4.3.2 Effects of drying on functional and nutritional properties

The bile salt binding capacity (BSBC) and water holding capacity (WHC) significantly decreased in heat dried samples. In general, the freeze-dried samples retained the highest BSBC and WHC, followed by the air-dried samples and the drum-dried samples, respectively (Table 4.6).

The BSBC decreased in the blanched pulps (Table 4.6) while the SDF increased and the TDF remained stable (Table 4.5). This could be because the heat-dried blanched samples were exposed to a more severe heat treatment, i.e., both blanching and drying, than the heat-dried un-blanched samples. Though the TDF was not affected, the BSBC decreased due to the more severe physical changes in blanched pulps and the higher depolymerization of the dietary fiber (DF), which led to its lower molecular weight, resulting in its lower viscosity.

Robertson and Eastwood (1981) suggested that WHC is considered to be a function of fiber structure rather than chemical composition. The method of drying was reported to affect the fiber structure, which is related to the changes in WHC. They reported the WHC of air-dried and freeze-dried potatoes to be 10.3 and 25.2 g/g, respectively, while their dietary fiber contents were only slightly different. They also observed the compression of cellular appearance in air-dried sample.

During thermal drying, plant tissues undergo a change of structure due to the solute redistribution and shrinkage (Fellows, 1988). The bulk density and porosity of dried vegetables depends on the drying conditions. With high initial drying rate, the surfaces of vegetables increase their rigidity and their final volume is fixed early in the drying process. As drying continues, the splitting and rupture of internal tissues yield

Table 4.6: Nutritional properties of dried frozen rhubarb pulps (dry weight basis)

Attributes	FDRP[1]		ADRP[2]		DDRP[3]	
	Un-blanched	Blanched	Un-blanched	Blanched	Un-blanched	Blanched
BSBC[4]	19.49 ± 0.40^{a}	18.43 ± 0.80^{a}	14.10 ± 1.13^{b}	12.47 ± 0.67^{c}	14.40 ± 0.60^{b}	11.46 ± 0.75^{c}
ORAC[5] (μmoleTE/g)	108	116	148	-	164	-
Water holding capacity (g/g)	16.66 ± 0.28^{a}	14.39 ± 0.15^{b}	10.46 ± 0.12^{d}	11.12 ± 0.12^{c}	9.64 ± 0.22^{e}	9.16 ± 0.23^{f}

Values are means ± SD. Values, presented in the same row, not sharing a common superscript letter are significantly different at $p < 0.05$.
[1] FDRP = Freeze-dried rhubarb pulp; [2] ADRP = Aired dried rhubarb pulp; [3] DDRP = Drum dried rhubarb pulp; and [4] BSBC = Bile salt binding capacity (* μmole Taurocholate/100mg fiber)
[5] ORAC = Oxygen Radical Absorption Capacity expressed as micromole Trolox equivalent (TE) per gram or litter.

opening cavities. The product obtained from this method has a low bulk density and good rehydration property. Drying with low initial rate, on the other hand, gives the product a high bulk density and poor rehydration property (Brennan *et al.*, 1969).

The degree of polymerization and the viscosity of fiber were highly dependent on the type of cooking and degree of heat treatment. Time and intensity of heat treatment are crucial for the splitting of glycosidic linkages. Svanberg *et al.* (1995) reported that freezing of carrot gave soluble fiber the highest degree of polymerization (DP), while blanching and microwave heating gave the soluble fiber an intermediate DP, and boiling gave the lowest DP. The viscosity of water soluble polysaccharides was also affected by type of processing in the same way.

The antioxidant capacities of air-dried and drum-dried rhubarb pulps, reported in ORAC units, were higher than that of freeze-dried sample. This could be due to the formation of Maillard's reaction products (MRPs) in the air-dried and drum-dried samples (Nicoli *et al.*, 1997).

The total antioxidant capacity of drum-dried sample was higher than that obtained from air-drying. This may be attributed to the effect of moisture content during drying as explained previously (Section 4.3.1). Fellows (1988) stated that lowering moisture content increases the rate of Maillard's reaction, which, in turn, increases the amount of non-nutritive antioxidants.

4.4 Effects of thermal concentration on rhubarb juice

Concentration of food juices leads to the decrease of their volume and weight, resulting in the reduction in storage space and distribution cost. This process also helps preserve the food products by reducing their water activity.

Before concentration by vacuum evaporation, oxalic acid was removed from rhubarb juice, using calcium chloride treatment established by Cai *et al.* (2000). The procedure was described in Chapter 3 (Section 3.3.2).

The composition and nutritional properties of fresh and frozen rhubarb juice at different concentrations were analyzed and the following are the results.

4.4.1 Effects of thermal concentration on composition

The soluble dietary fiber (SDF) content decreased when rhubarb juice was subjected to the concentration process. Similarly, total anthocyanin levels in concentrated samples were lower than the one present in raw juice (Table 4.7). The results of frozen samples showed the same trends as those of the fresh samples (Table 4.8).

The decrease of soluble dietary fiber in concentrated juice could be partly due to the activity of enzyme pectin methyl esterase (PME) which was naturally inactivated in living plant tissue, but was activated when the tissue was damaged by heating or freezing (Van Buren, 1979). More likely, it was largely due to the depolymerization of the SDF during the thermal concentration process.

Table 4.7: Results of analysis of some attributes of fresh rhubarb juices at different concentrations

Attributes	Fresh juice (3.36% TSS)	Concentrated juice		
		50% TSS[1]	65% TSS	75% TSS
pH	3.15	1.58	1.27	0.99
Insoluble dietary fiber (%)*	0.00	0.00	0.00	0.00
Soluble dietary fiber (%)*	1.32 ± 0.04^{a}	0.36 ± 0.02^{b}	0.33 ± 0.00^{b}	0.25 ± 0.00^{b}
Total dietary fiber (%)*	1.32 ± 0.04^{a}	0.36 ± 0.02^{b}	0.33 ± 0.00^{b}	0.25 ± 0.00^{b}
TACN[2] (mg/L)*	106.03 ± 0.78^{a}	94.21 ± 0.31^{b}	92.03 ± 0.42^{c}	90.54 ± 0.44^{d}
TACN[2] (mg/L)* after frozen storage for 3 months	17.79 ± 1.23^{d}	52.00 ± 1.40^{c}	71.99 ± 3.20^{b}	85.97 ± 1.59^{a}
Protein content (%)	0.00	0.00	0.00	0.00
Fat content (%)	0.00	0.00	0.00	0.00
ORAC[3] (μmoleTE/L)	2,509	1,741	-	-

Values are means ± SD. Values, presented in the same row, not sharing a common superscript letter are significantly different at $p < 0.05$.

* present at the 3% TSS

[1] TSS = Total soluble solids

[2] TACN = Total anthocyanin (1 L of sample was extracted from 500 g of dried rhubarb pulp)

[3] ORAC = Oxygen Radical Absorption Capacity expressed as micromole Trolox equivalent (TE) per gram or litter.

Table 4.8: Results of analysis of some attributes of frozen rhubarb juices at different concentrations

Attributes	Frozen juice (3.85% TSS)	Concentrated juice		
		50% TSS[1]	65% TSS	75% TSS
pH	3.22	1.53	1.28	1.01
Insoluble dietary fiber (%)*	0.00	0.00	0.00	0.00
Soluble dietary fiber (%)*	0.90 ± 0.20^{a}	0.32 ± 0.02^{b}	0.23 ± 0.01^{b}	0.21 ± 0.01^{b}
Total dietary fiber (%)*	0.90 ± 0.20^{a}	0.32 ± 0.02^{b}	0.23 ± 0.01^{b}	0.21 ± 0.01^{b}
TACN[2] (mg/L)*	105.94 ± 0.80^{a}	84.61 ± 0.40^{b}	82.69 ± 0.32^{c}	81.65 ± 0.41^{d}
TACN[2] (mg/L)* after frozen storage for 3 months	17.93 ± 0.47^{d}	44.97 ± 0.25^{c}	62.39 ± 2.08^{b}	80.40 ± 0.99^{a}
Protein content (%)	0.00	0.00	0.00	0.00
Fat content (%)	0.00	0.00	0.00	0.00

Values are means $\pm$ SD. Values, presented in the same row, not sharing a common superscript letter are significantly different at $p < 0.05$.
* present at the 3% TSS
[1] TSS = Total soluble solids
[2] TACN = Total anthocyanin (1 L of sample was extracted from 500 g of dried rhubarb pulp)

The amount of anthocyanin pigments in the juice, measured at 3% TSS immediately after the evaporation process, decreased as the concentration of the sample increased. This indicated thermal degradation of the anthocyanin in the juice due to the mild heat during vacuum evaporation. The conversion of leucoanthocyanin to anthocyanin could also happen, but probably at a slower rate than that of thermal degradation.

After 3 months of storage at -20°C, the analytical results showed that the concentrated rhubarb juices could retain their anthocyanins better than raw juice (Tables 4.7 and 4.8, and Fig. 4.3). Thus the stability of anthocyanins in the juice improved as its concentration increased. This can be attributed to a number of factors.

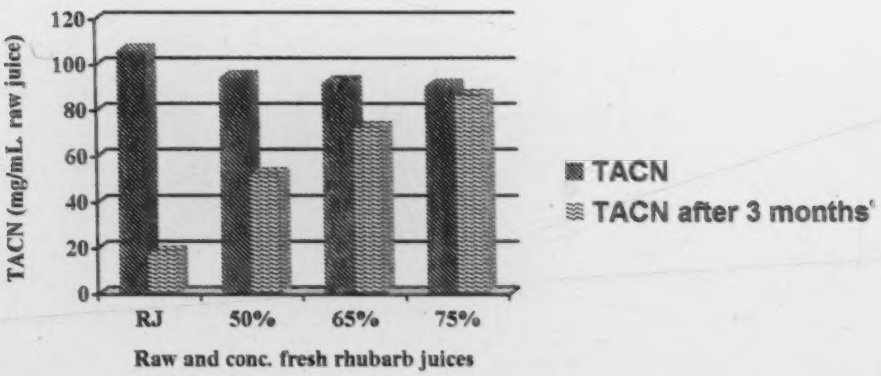

Figure 4.3: Total anthocyanin contents in raw and conc. fresh rhubarb juices at 3% TSS
TSS = Total soluble solids; TACN = Total anthocyanin; and RJ = Raw juice

In water, for most anthocyanins, the only stable colored species is the flavylium ion, which is generally obtained at pH values lower than 3 (Brouillard, 1988). At pH ~1, acidic solutions always quantitatively regenerate the flavylium cation, if there is any reversible condition that would allow the cleavage of pyrylium ring, such as exposure the pigments to alkaline solutions. Therefore, the lower pH of concentrated rhubarb juice helped maintain the TACN in the concentrated juice during storage.

Although the temperature the juice was exposed to during vacuum evaporation was not as high as that in atmospheric evaporation, the anthocyanin degradation could still occur through enzymatic activities since the enzymes may only be partially destroyed by heat during the concentration process.

In aqueous system, anthocyanins usually condense with themselves (self-association) and with other organic compounds (co-pigmentation) (Markakis, 1974). The stability of all of these colored complexes under conditions encountered in the processing has not been systematically studied. It appears, however, that these complexes are not as

labile as most of the uncondensed anthocyanins (Timberlake, 1980; Markakis, 1974). Asen *et al.* (1972) and Jurd and Asen (1966) reported that the concentration of anthocyanins was a limiting factor in co-pigmentation, hence the condensation of anthocyanins is prone to occur in concentrated solution at a higher level than in the diluted raw juice. Mazza and Brouillard (1990) suggested that pH is also a factor affecting co-pigmentation of anthocyanins. The stability of anthocyanin pigments increased as the pH decreased in the pH range of 1.0-5.0 (Meschter, 1953).

4.4.2 Effects of thermal concentration on nutritional properties

The concentrated juice showed lower antioxidant capacity compared to the raw juice, though the TACN levels of the three months old frozen juices showed the opposite trend (Table 4.7). This could imply that anthocyanin is not the main component providing antioxidant activity in rhubarb juice. The compounds that play a major role are probably the water-soluble and heat-labile species, such as vitamin C, which is well known in its antioxidative power (Ou *et al.*, 2002 and Larson, 1988). Additionally, the antioxidative enzymes, such as catalase, peroxidase and superoxide dismutase (Larson, 1988), may only be partly inactivated during the evaporation process.

According to the ORAC Vegetable Chart provided by Brunswick Laboratories (2003), purple cabbage presented the highest total antioxidant value (42 μmoleTE/g fresh weight basis) following by kale (37 μmoleTE/g fresh weight basis). Other vegetables such as tomato, carrot, red pepper and spinach showed the activity between 3-24 μmoleTE/g fresh weight basis. From the ORAC Fruit Chart, black raspberry demonstrated the highest total antioxidant value (164 μmoleTE/g fresh weight basis) and

several fruits such as apple, orange and boysenberry had their ORAC Value in the range of 14-61μmole TE/g fresh weight basis.

The approximate total antioxidant capacity of 'Macdonald' rhubarb calculated from the data of freeze-dried frozen pulp, and frozen rhubarb juice, based on the proportion of dried pulp:juice = 7:93 is 9.89 μmoleTE/g fresh weight. Thus, rhubarb may be categorized as a plant exhibiting low antioxidant capacity.

4.5 Experiments on antimicrobial property of rhubarb

The alcohol and water extracts from dried pulps, as well as fresh and frozen rhubarb juices, did not show any antimicrobial effect on selected microorganisms, i.e. *Bacillus subtilis*, *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Salmonella typhimurium* and *Staphylococcus aureus* as shown in Table 4.9. However, the concentrated juice did cause clear zones in all microorganisms tested.

Rhubarb's antibacterial activity against *S. aureus*, *B. abortus*, *E. coli*, *B. subtilis* and *Clostridium perfringens* was observed by several researchers (Chopra and Nayyar, 1978; and Ahmad *et al.*, 1998). Ahmad *et al.* (1998) suggested that this property of rhubarb could be due to rhein, one of the common anthraquinones in most of the rhubarb species.

However, the clear zones occurred when tested on the selected microbial cultures with concentrated rhubarb juices may be attributed to the effect of their acidity as well.

The survival of microorganisms depends on several parameters, one of which is pH of the environment. All microorganisms have their individual range of pH for growth. Generally, bacteria will tolerate a range of pH 4-9 (Doores, 1983).

Table 4.9: Results of antimicrobial activity of rhubarb pulp and juice tested on some microorganism

Sample extract*	pH	Clear zone (cm.)				
		E. *coli*	S *aureus*	B. *cereus*	S. *typhimerium*	L. *monocytogenes*
Fs/FDRP[1]	2.315	0.00^{c}	0.00^{d}	0.00^{d}	0.00^{b}	0.00^{c}
Fz/FDRP[2]	2.351	0.00^{c}	0.00^{d}	0.00^{d}	0.00^{b}	0.00^{c}
Fz/ADRP[3]	2.338	0.00^{c}	0.00^{d}	0.00^{d}	0.00^{b}	0.00^{c}
Fz/DDRP[4]	2.363	0.00^{c}	0.00^{d}	0.00^{d}	0.00^{b}	0.00^{c}
Rhubarb juice	2.107	0.00^{c}	0.00^{d}	0.00^{d}	0.00^{b}	0.00^{c}
50% TSS RJ[5]	1.526	8.25 ± 0.29^{b}	9.75 ± 0.50^{c}	9.00 ± 0.00^{c}	9.63 ± 0.48^{a}	13.38 ± 1.11^{b}
65% TSS RJ[5]	1.283	8.75 ± 0.29^{a}	12.5 ± 0.58^{b}	9.25 ± 0.29^{b}	9.75 ± 0.87^{a}	15.00 ± 0.91^{a}
75% TSS RJ[5]	1.008	8.75 ± 0.29^{a}	13.88 ± 1.1^{a}	10.00 ± 0.00^{a}	10.25 ± 0.29^{a}	15.25 ± 0.65^{a}

Values are means ± SD. Values, presented in the same column, not sharing a common superscript letter are significantly different at $p < 0.05$.
* Only the results of alcohol extract of dried un-blanched rhubarb pulps, and data obtained from concentrated and raw fresh rhubarb juices are presented in the table
[1] Fs/FDRP = Freeze-dried fresh rhubarb pulp; [2] Fz/FDRP = Freeze-dried frozen rhubarb pulp; [3] Fz/ADRP = Air-dried frozen rhubarb pulp; [4] Fz/DDRP = Drum-dried frozen rhubarb pulp; and [5] RJ = Rhubarb juice

To observe the effect of pH of the extracts on tested bacteria, mixtures of malic and citric acids at different pHs similar to the pHs of rhubarb extracts and juices were used. The results presented in Table 4.10 showed that clear zones appeared in every microorganism treated with the mixtures at pHs 0.998, 1.110, 1.198 and 1.575, which represented the pH of concentrated rhubarb juices. At pHs 2.100, 2.500 and 3.001, which are similar to the pH of water and alcohol extracts of dried rhubarb pulps, the acid mixtures did not cause clear zones in any of the selected bacteria.

This can possibly imply that the effect of antibacterial activity in concentrated rhubarb juices is largely due to the acidity of the juices.

At the same pH, the clear zones caused by acid mixtures were larger than those caused by concentrated rhubarb juice. This could be due to the form of acids present in the juice samples, i.e. they may be bound to other components, making them unavailable to exert the antimicrobial property.

Also, microorganisms show varied tolerance to different kinds of acids. For example, acetic acid can be harmful to the metabolism of lactobacilli, but inhibitory to bacilli. Apart from malic and citric acids, the presence of palmitic, acetic, succinic, lactic, fumaric and formic acids in rhubarb was also reported (Agarwal *et al.*, 2001). Therefore, the combination of these acids in rhubarb juice may give the different results, i.e. the different sizes of the clear zones as compared to the clear zones caused by malic and citric acids in selected bacteria.

Table 4.10: Results of antimicrobial activity of rhubarb pulp and juice tested on some microorganism

pH of malic and citric acid solution	Clear zone (cm.)				
	E. *coli*	S *aureus*	B. *cereus*	S. *typhimerium*	L. *monocytogenes*
0.998	16.25 ± 0.35^{a}	18.00 ± 2.12^{a}	18.75 ± 0.35^{a}	15.00 ± 0.71^{a}	24.25 ± 1.06^{a}
1.110	15.25 ± 0.35^{b}	15.50 ± 2.12^{ab}	13.00 ± 0.71^{b}	14.25 ± 1.06^{a}	22.75 ± 0.35^{b}
1.198	12.75 ± 0.35^{c}	13.25 ± 0.35^{b}	11.25 ± 0.35^{c}	12.50 ± 0.71^{b}	20.00 ± 0.00^{c}
1.575	12.00 ± 0.71^{c}	10.00 ± 0.71^{c}	10.00 ± 0.00^{d}	10.00 ± 0.00^{c}	14.75 ± 0.35^{d}
2.100	0.00^{d}	0.00^{d}	0.00^{e}	0.00^{d}	0.00^{e}
2.500	0.00^{d}	0.00^{d}	0.00^{e}	0.00^{d}	0.00^{e}
3.001	0.00^{d}	0.00^{d}	0.00^{e}	0.00^{d}	0.00^{e}

Values are means $\pm$ SD. Values, presented in the same column, not sharing a common superscript letter are significantly different at $p < 0.05$.

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CHAPTER 5

General conclusions and suggestions for further research

Processing treatments such as freezing, blanching, drying and evaporation have been shown to affect chemical components such as dietary fiber and anthocyanins in rhubarb pulp and juice. This, in turn, affects their functional and nutritional properties such as water holding, bile salt binding, total antioxidant, and antimicrobial capacities.

Storage at -20°C caused cell shrinkage in rhubarb stalks (Mohr, 1971) leading to damaged cell wall, causing drip loss (Brown, 1979). Freezing and thawing of the stalks facilitated the separation of juice from pulp in the pressing operation. However, this resulted in some loss of soluble dietary fiber (SDF), total anthocyanins (TACN), protein and fat from the pulp, while its insoluble dietary fiber (IDF) and ash contents remained unchanged. The decrease in SDF and the change in cell structure of the pulp reduced its ability to bind bile salt and trap water when the frozen rhubarb pulp was dried (Robertson and Eastwood, 1981).

Blanching also decreased IDF, protein and ash contents in rhubarb pulp. However, the process increased SDF and TACN contents while fat remained unchanged. These indicated that blanching caused the degradation and solubilisation of pectin in fiber matrix (Gooneratne *et al.*, 1994).

On the other hand, blanching helped inactivate enzymes in rhubarb pulp resulting in a better retention of anthocyanin as compared to the unblanched pulp (Wrolstad *et al.*, 1980). Heat from blanching could also convert leucoanthocyanin pigments to anthocyanin (www1).

Although the heat treatment increased the soluble portion of dietary fiber (Svanberg *et al.*, 1995), the depolymerization of readily soluble fiber decreased its molecular weight resulting in the decrease in its viscosity (Van Buren, 1979). These, combined with the disruption of cell walls occurred during the blanching process (Gooneratne *et al.*, 1994, and Mohr, 1971), led to the decline in water holding capacity (WHC) and bile salt binding capacity (BSBC) in blanched samples (Robertson and Eastwood, 1981).

Antioxidant capacity of the blanched samples increased due to the increase in total anthocyanins, and, possibly, the formation of Maillard's reaction products (MRPs) (Nicoli *et al.*, 1997).

Drying processes involving high temperatures, such as air- and drum-drying, lowered total dietary fiber (TDF), TACN, and protein contents in rhubarb pulps. The loss of fiber fractions via dextrinization and thermal decomposition was possible (Fornal *et al.*, 1987). Fat concentration in all samples remained unchanged, which could be because it consisted principally of saturated fatty acids (Nawar, 1985). The reduction of anthocyanins could be attributed to the exposure of the samples to heat (Markakis, 1974)

Air-dried samples retained a higher anthocyanin concentration compared to drum-dried samples. This may be due to the difference in drying temperatures and moisture contents of the samples during drying by the two methods.

As compared to freeze-dried samples, BSBC and WHC in air- and drum-dried samples decreased significantly, with a higher capacity in air-dried pulp than in drum-dried pulp.

In the thermal drying processes, plant tissues undergo structural changes (Fellows, 1988). The heat also caused depolymerization of fiber fractions resulting in the decrease in viscosity of the fiber (Svanberg *et al.*, 1995). These led to the poor WHC and BSBC in samples dried by thermal drying as compared to the freeze-dried sample.

Blanching and thermal drying of rhubarb pulp led to a more severe physical alteration and higher degree of polymerization of dietary fiber in dried pulps as compared to the dried un-blanched samples, thus lowering their BSBC and WHC further than drying alone.

On the other hand, thermal drying increased the samples' antioxidant capacity, with the higher capacity in drum-dried pulp, possibly due to the formation of Maillard's reaction products (MRPs) (Nicoli *et al.*, 1997).

After concentration, the SDF and TACN in concentrated rhubarb juice, expressed from both fresh and frozen rhubarb stalks, decreased as compared to those in raw juice. The heat applied during the process could cause the depolymerization of the SDF as well as the thermal degradation of the anthocyanin in the juice.

The concentrated rhubarb juices showed higher stability in their anthocyanin contents than raw juice after three months of frozen storage (-20°C). The stability of anthocyanins in the juice improved as its concentration increased (Asen *et al.*, 1972; Brouillard, 1988 and Markakis, 1974).

Although the total anthocyanin levels of the three months old frozen concentrated juices were higher than that in the raw juice, the concentrated juice showed a lower antioxidant capacity. This probably indicated that anthocyanin is not the main component providing antioxidant activity in rhubarb juice. Instead, the major active antioxidants may

be the water-soluble and heat-labile compounds, such as vitamin C and the antioxidative enzymes (Larson, 1988).

The approximate total antioxidant capacity of 'Macdonald' rhubarb was 9.89 μmole TE/g fresh weight. Therefore, rhubarb may be categorized as a plant possessing a low antioxidant capacity (Brunswick Laboratories, 2003)

Both water and alcohol extracts from rhubarb pulp, as well as raw fresh and frozen juice, did not show any antimicrobial activity against *B. subtilis*, *E. coli* O157:H7, *L. monocytogenes*, *S. typhimurium* and *S. aureus*. On the other hand, concentrated juice did cause clear zones in all microorganisms tested. However, the clear zones may be attributed mainly to the effect of their acidity (Doores, 1983) as the mixtures of malic and citric acids at different pHs similar to the pHs of rhubarb extracts and juices exhibited similar results.

The investigation of the effects of processing treatments, i.e. freezing, blanching, drying and evaporation on chemical, functional and nutritional properties of rhubarb pulp and juice in this project has extended our scientific and technological understanding on this valuable but underutilized crop. It will certainly help toward the attempt to utilize and develop rhubarb as food ingredients and products. However, additional work is still needed to make the attempt a reality.

For further development, the following additional research is recommended:

1. Development of suitable processing methods, with the optimum operating conditions, to preserve rhubarb's nutritional and functional attributes, such as BSBC and WHC, and enhance some properties, such as antioxidant capacity, at the same time.

2. Identification of major contributors to the antioxidant capacity in rhubarb juice, which may lead to the development of its suitable preservation methods.
3. Investigation of the relationship between chemical, nutritional, functional as well as sensory properties of rhubarb, such as the effect of viscosity of soluble dietary fiber on WHC and BSBC and the effect of anthocyanin level on color of rhubarb.

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Appendices

Appendix 1: Two pathways of anthocyanin degradation (Markakis, 1974)